CLINICAL PHARMACOLOGY and THERAPEUTICS

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Clinical Pharmacology

and Therapeutics

Editorial Communications

Clinical Pharmacology and Therapeutics is a bimonthly journal devoted to the publication of original articles and reviews dealing with the effects of drugs in man. Articles are accepted on the condition that they are contributed solely to *Clinical Pharmacology and Therapeutics*. Neither the editors nor the publisher accept responsibility for the views and statements of authors expressed in their communications. Manuscripts submitted for publication should be sent to the Editor.

Manuscripts. Manuscripts should be typewritten on one side of the paper only, with double spacing and liberal margins. Manuscripts should be submitted in duplicate, i.e., an original and one carbon copy. Failure to submit a duplicate copy prolongs editorial decisions considerably.

Summaries. A summary of not more than 250 words must also be submitted on a separate sheet in duplicate. It will appear under the title of the published article.

References. References should be placed at the end of the article (not as footnotes on each page), listed alphabetically and numbered accordingly. They should conform to the style of the Quarterly Cumulative Index Medicus and include complete pagination. Personal communications should be excluded from the references and used, if at all, only as footnotes. In press references may be used only if the accepting journal is indicated.

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A reasonable number of halftone illustrations will be reproduced free of cost to the author, but special arrangements must be made with the Editor for color plates, elaborate tables, or extra illustrations. Copy for zinc cuts (such as pen drawings and charts) must be drawn in India ink and lettered in India ink or black typewriter ribbon. Only good photographic prints and original drawings should be supplied for halftone work.

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Review of Books. Books and monographs, native and foreign, will be reviewed according to their merits and as space permits. Books may be sent to Dr. Walter Modell, 1300 York Avenue, New York 21, New York.

American Therapeutic Society. Official notices of the Society will appear in the Journal.

Drug Nomenclature. Only generic and chemical names of drugs are permitted. A proprietary equivalent may be indicated *once*, in a footnote.

Business Communications

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Unless your practice is limited to bacteriology ... or your patients are all in the upper income brackets...you have doubtless received complaints about the cost of the medication you prescribe.

what your patient gives...and gets

Some of these complaints can probably be dismissed lightly as coming from cranks, who would complain about your fee for a midnight house call to save the life of a dying child. Others, however, are made seriously by thoughtful patients and deserve an answer in kind. You know what the patient gets from his pharmacist because you have prescribed it. Do you also know that the average cost of a prescription is about $3.00? Only about one in 100 costs $10.00 or more, and 3 out of 5 of the prescriptions are under $3.00. These figures are based on retail prices. They include the manufacturer's research, development, and manufacturing costs and all distribution costs of the wholesale and the retail druggist. Only you and your patients can judge whether today's drugs at these prices represent a fair *quid pro quo*, an equitable balance between what is given and what is received.

Erythropoietin—a significant discovery in clinical hematology

A wealth of evidence now confirms the fact that red blood cell production is controlled by the hormone erythropoietin.[1-3] Demonstrated in human plasma,[4] erythropoietin has been shown to produce reticulocytosis,[1,5-7] increase utilization of the Fe^{59} isotope, and increase erythrocyte precursors in marrow cultures.[3,8]

ERYTHROPOIETIN FOUND TO CONTROL RED CELL FORMATION

erythropoietin levels—new criteria in diagnosis of anemia — Increased erythropoietin blood levels can be demonstrated in severe anemia and following the start of accelerated formation.[9] Soon thereafter, the effect of the higher levels appears as an increased erythroid marrow activity.[10] Since the hemopoietic marrow is capable of producing more red cells than normally required, many anemias may be due to inadequate erythropoietin levels—a result of subnormal production or excessive excretion.

how does erythropoietin affect iron metabolism? Absorption and utilization of iron are dependent upon the rate of bone marrow erythropoiesis which, in turn, is dependent upon erythropoietin levels.[11,12] Thus, the demand for iron created by accelerated erythropoiesis is satisfied by both increased gastrointestinal absorption and mobilization of storage iron. Inadequate erythropoietin levels would seemingly account for the frequently disappointing results with the use of iron alone in many of the anemias.

can medication increase erythropoietin levels? Cobalt has been shown to be strikingly effective in increasing the production of erythropoietin.[13,14] Cobalt-enhanced erythropoietin accelerates red cell production and improves iron utilization with a subsequent increase in hemoglobin and erythrocytes. The new concepts of the cause, diagnosis, and management of anemia may now be applied clinically on the sound basis of extensive studies published on RONCOVITE®—MF,*the therapeutic cobalt-iron hematinic.

(1) Gordon, A. S.: Physiol. Rev. **39**:1, 1959. (2) Erslev, A. J.: J. Lab. & Clin. Med. **50**:543, 1957. (3) Rosse, W. F., and Gurney, C. W.: J. Lab. & Clin. Med. **53**:446, 1959. (4) Gurney, C. W.; Goldwasser, E., and Pan, C.: J. Lab. & Clin. Med. **50**:534, 1957. (5) Rambach, W. A.; Alt, H. F., and Cooper, J. A. D.: Blood **12**:1101, 1957. (6) Gordon, A. S., et al.: Proc. Soc. Exp. Biol. & Med. **92**:598, 1956. (7) Erslev, A. J.: Blood **10**:954, 1955. (8) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L. F.: Blood **13**:55, 1958. (9) Stohlman, F., Jr., and Brecher, G.: Proc. Soc. Exp. Biol. & Med. **100**:40, 1959. (10) Kraus, L. M., and Kraus, A. P.: Fed. Proc. **18**:1051, 1959. (11) Bothwell, T. H.; Pirzio-Biroli, G., and Finch, C. A.: J. Lab. & Clin. Med. **51**:24, 1958. (12) Beutler, E., and Buttenwieser, E.: J. Lab. & Clin. Med. **55**:274, 1960. (13) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L.: Science **125**:1085, 1957. (14) Murdock, H. R., Jr., and Klotz, L. J.: J. Am. Pharm. A. (Scient. Ed.) **48**:143, 1959.

*Cobalt chloride (cobalt as Co 3.7 mg.), 15 mg. ferrous sulfate exsiccated, 100 mg.

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Cyclophosphamide, Mead Johnson

for palliative chemotherapy of
certain types of malignant neoplasms

"Cyclophosphamide [Cytoxan] has proved a valuable addition to chemotherapeutic drugs available for the treatment of malignant diseases of the haemopoietic and reticuloendothelial systems.... Particularly effective in Hodgkin's disease, lymphosarcoma, chronic lymphocytic leukaemia, and myelomatosis...."[1]

"Objective data suggest that this agent [Cytoxan] has advantages not possessed by standard alkylating agents now in clinical use."[2]

"With the use of cyclophosphamide [Cytoxan] there is a relative lack of thrombocytopenia and a diminution in gastrointestinal side-effects, so that it may offer therapeutic advantages over other alkylating agents."[3]

Other Advantages in Clinical Practice: Broad-spectrum application. High therapeutic index. No vesicant activity—may be given orally or parenterally.

(1) Matthias, J. Q.; Misiewicz, J. J., and Scott, R. B.: Brit. M. J. 2:1837-1840 (Dec. 24) 1960.
(2) Coggins, P. R.; Ravdin, R. G., and Eisman, S. H.: Cancer *13*:1254-1260 (Nov.-Dec.) 1960.
(3) Papac, R.; Petrakis, N. L.; Amini, F., and Wood, D. A.: J.A.M.A. *172*:1387-1391 (March 26) 1960.

DOSAGE: *For neoplasms relatively susceptible to Cytoxan* — Patients with lymphomas and other neoplasms believed to be relatively susceptible to Cytoxan therapy are given an initial dose of 2-3 mg./Kg./day intravenously. White blood counts and platelet determinations should be made daily or twice weekly and the dosage adjusted accordingly. Intravenous infusions should be continued for at least 6 days unless otherwise indicated. A leukopenia of between 1500 and 5000 cells per cu. mm. (or lower) may be expected between the tenth and fourteenth day. In the presence of a leukopenia of less than 2000/cu. mm. Cytoxan should be discontinued until the white cell count returns to 2000 to 5000 (usually within a week). Dosage is subsequently adjusted as indicated by the patient's objective response and the leukocyte count. If the patient is subjectively improved, if the size of the tumor has decreased, or if the white cells are satisfactorily maintained between 2000 and 5000/cu. mm. oral dosage may be instituted equivalent to intravenous dosage.

Thrombocytopenia is rarely observed on this regimen. If platelet counts of less than 100,000/cu. mm. are observed, the patient should be watched carefully. If platelets continue to decrease, Cytoxan should be discontinued.

The patient who has had previous treatment with alkylating agents, or x-ray, or is debilitated may be more susceptible to bone marrow depression, and initial Cytoxan doses should be more conservative than the above. Such patients should have more frequent hematologic evaluation. Good medical practice demands access to a reliable hematologic laboratory when using Cytoxan.

For neoplasms relatively resistant to Cytoxan — Patients with carcinomas and other malignant neoplasms believed to be less susceptible to Cytoxan therapy are given a dose of 4 to 8 mg./Kg./day intravenously. Unless there are indications to the contrary, this dose is continued for 6 days, then stopped. Leukopenia usually ensues on the tenth to fourteenth day after the first dose of Cytoxan. Thrombocyte reduction is not common, and platelets may actually increase. The leukocyte count promptly returns toward normal levels in most cases, and as it begins to increase, sufficient Cytoxan is administered to maintain it near 2000 to 5000/cu. mm. This may be accomplished by two intravenous injections weekly, or by oral administration, or by a combination of both routes. An oral dosage of 50 to 200 mg. daily or an intravenous injection of 5 mg./Kg. twice weekly will usually suffice.

The platelet and leukocyte counts should be followed carefully, and the prior treatment history of patients carefully evaluated as delineated above.

Leukopenia as a guide to adequacy of dosage — The best objective measure for dosage seems to be the number of circulating white blood cells. This is used as an index of the activity of the hematopoietic system, especially the bone marrow. The mechanism by which Cytoxan causes a reduction in the level of white blood cells is not known, but cessation of dosage results in an increase in the level, indicating that the hematopoietic system had not been permanently affected. When large doses (8 mg./Kg./day for 6 days) are given initially, the white cell count falls rapidly. Following the cessation of the 6-day course, the white cells may continue to decline for as long as 8 days and then increase. The reduction of the white cell count during Cytoxan therapy and its subsequent increase when therapy is discontinued can be repeated in the same patient. Maximal reduction in leukocyte count indicates the maximal permissible Cytoxan level for therapeutic effect. Leukopenic patients must be watched carefully for evidence of infection.

Total white blood cell and thrombocyte counts should be obtained 2 or more times weekly in order to evaluate therapy and to adjust dosage.

SIDE EFFECTS: Although Cytoxan is related to nitrogen mustard, it has no vesicant effect on tissue. It does not traumatize the vein when injected intravenously, nor does it cause any localized tissue reaction following extravasation. It may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally or directly into the tumor, when indicated. It is apparently active by each of these routes.

Nausea and vomiting are common and depend on dose and on individual susceptibility. However, many investigators accept the nausea and vomiting in favor of maintaining maximal therapy. The vomiting can be controlled with antiemetic agents.

Alopecia is a frequent side reaction to Cytoxan therapy. It has been observed in 28% of the patients studied in this country. The incidence is greater with larger doses. The loss of hair may first be noted about the 21st day of therapy and may proceed to alopecia totalis. This effect is reversed following discontinuance of Cytoxan; during reduced maintenance therapy, hair may reappear. It is essential to advise the patient in advance concerning this effect of the drug.

Dizziness of short duration and of minor degree has occasionally been reported.

Leukopenia is an expected effect and can be used as a guide to therapy. Thrombocytopenia may occur, especially after large doses. The leukocyte or platelet counts of an occasional patient may fall precipitously after even small doses of Cytoxan, as with all alkylating agents. The drug should be discontinued in such patients and reinstituted later at lower dosage after satisfactory hematologic recovery has occurred. Prior treatment with x-ray or with other chemotherapeutic agents frequently causes an earlier or exaggerated leukopenia or thrombocytopenia after Cytoxan medication. Only rarely has there been a report of erythrocyte or hemoglobin reduction.

ADMINISTRATION: Add 5 cc. sterile water (Water for Injection, U.S.P.) to 100 mg. of Cytoxan in the sterile vial (add 10 cc. to 200 mg. vial). Shake, allow to stand until clear, remove with sterile syringe and needle and inject.

The freshly prepared solution of Cytoxan may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally, or directly into the tumor. The solution should be administered promptly after being made but is satisfactory for use for three hours after preparation.

If the patient is receiving a parenteral infusion, the Cytoxan solution may be injected into the rubber tubing if the solution is glucose or saline.

No thrombosis or thrombophlebitis has been reported from injections of Cytoxan. Extravasation of the drug into the subcutaneous tissues does not result in local reactions.

PRECAUTIONS: Cytoxan should not be given to any person with a severe leukopenia, thrombocytopenia, or bone marrow infiltrated with malignant cells. It may be given with suitable precautions to patients who have had recent x-ray treatment, recent treatment with a cytotoxic agent, a surgical procedure within 2-3 weeks, or debilitated patients.

AVAILABILITY: Cytoxan is available as follows:

Cytoxan for Injection, 100 mg., a sterile dry-filled vial containing 100 mg. cyclophosphamide and 45 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan for Injection, 200 mg., a sterile dry-filled vial containing 200 mg. cyclophosphamide and 90 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan Tablets for oral administration, 50 mg., white, round tablets, flecked with blue for easy identification. Packaged, 100 tablets per bottle.

For a copy of the Cytoxan brochure, or other additional information on Cytoxan, communicate directly with the Medical Department, Mead Johnson Laboratories, Evansville 21, Indiana.

when allergies separate a man from his work...

Florists may develop allergies to flowers, insecticides and Holland bulbs...housewives to dust and soap...farmers to pollens and molds. All types of allergies — occupational, seasonal or occasional reactions to foods and drugs — respond to Dimetane. With Dimetane most patients become symptom free *and* stay alert, and on the job, for Dimetane works ...with a significantly lower incidence[1-6] of the annoying side effects usually associated with antihistaminic therapy.

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parabromdylamine [brompheniramine] maleate

reliably relieve the symptoms...seldom affect alertness

Supplied: DIMETANE Extentabs®—12 mg. • DIMETANE Tablets—4 mg. • DIMETANE Elixir—2 mg./5 cc.

Dosage: *Extentabs:* Adults—One Extentab q. 8-12 h. or twice daily. Children over 6—one Extentab q. 12 h. *Tablets:* Adults—One or two tablets three or four times daily. Children over 6—one tablet t.i.d. or q.i.d. Children 3-6—½ tablet t.i.d. *Elixir:* Adults—2-4 teaspoonfuls t.i.d. Children over 6—2 teaspoonfuls t.i.d. or q.i.d. Children 3-6—1 teaspoonful t.i.d. Children under 3—0.5 cc. (0.2 mg.) per pound of body weight per 24 hours.

Side Effects: DIMETANE is usually well tolerated. Occasional mild drowsiness may be encountered. If desired, this may be offset by small doses of methamphetamine. Until known that the patient does not become drowsy, he should be cautioned against engaging in mechanical operations which require alertness.

Contraindications: Sensitivity to antihistamines. *Also Available:* Dimetane-Ten Injectable (10 mg./cc.) or Dimetane-100 Injectable (100 mg./cc.)

References: 1. Lineback, M.: The Eye, Ear, Nose and Throat Monthly *39*:342 (April) 1960. 2. Fuchs, A. M. and Maurer, M. L.: New York J. Med. *59*:3060 (August 15) 1959. 3. Kreindler, L. *et al.*: Antibiotic Med. and Clin. Therapy *6*:28 (January) 1959. 4. Schiller, I. W. and Lowell, F. C.: New England J. Med. *261*:478 (September 3) 1959. 5. Edmonds, J. T.: The Laryngoscope *69*:1213 (September) 1959. 6. Horstman, H. A.: Am. Pract. & Digest Treat. *10*:96 (January) 1959.

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...SEEKING TOMORROW'S WITH PERSISTENCE

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At a large teaching hospital, a double-blind study with FUROXONE LIQUID in 65 children "demonstrated both symptomatic and bacteriological effectiveness of this drug in the outpatient management of bacterial diarrhea" without eradication of the normal intestinal flora. This "highly desirable quality"—the preservation of normal intestinal flora in children—is held "in contrast to experience with other . . . agents used for this purpose." Overgrowth of nonsusceptible organisms "resulting in colitis, proctitis and anal pruritus usually associated with bowel sterilization have not been observed" with FUROXONE. "Side effects were negligible and acceptability of the preparation was excellent." [Mintz, A. A.: Antibiotic Med. 7:481, 1960.] **Furoxone Liquid** is a pleasant orange-mint flavored suspension containing Furoxone 50 mg. per 15 cc., with kaolin and pectin. Dosage for both children and adults may be found in your P.D.R.

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in any emetic situation
Tigan is safe and sound

Against nausea there's nothing more effective or, for that matter, quite so safe as Tigan. Tigan has **one** demonstrable pharmacologic property: it blocks emetic impulses, primarily by direct action at the chemoreceptor trigger zone (CTZ) of the medulla.[1] Tigan thus **stops** active vomiting as effectively as it prevents nausea and emesis.[2-5] Tigan is not a converted antihistamine, tranquilizer or sedative; it may be used even where older antiemetics are contraindicated.

USUAL DOSAGE

ADULTS: Capsules — The usual dosage is 100 mg to 250 mg, three or four times daily. In nausea and vomiting of pregnancy, one 250-mg capsule at bedtime and one immediately upon awakening. In severe cases this dose may be repeated up to a total of three or four capsules daily.

Intramuscular Injection — 200 mg (2 cc), one to four times daily.

Suppositories — 200 mg (one suppository), three or four times daily.

CHILDREN: Capsules — For children 30 to 90 pounds, one 100-mg capsule, three or four times daily.

Intramuscular Injection — Under 30 pounds, 50 mg (½ cc); 30 to 60 pounds, 100 mg (1 cc); 60 to 90 pounds, 150 mg (1½ cc) — one to four times daily.

Suppositories — Under 30 pounds, 100 mg (½ suppository); 30 to 90 pounds, 100 mg to 200 mg (½ to 1 suppository) — three to four times daily.

Note: There are no known contraindications or special precautions to be taken with Tigan therapy. Because Tigan has no sedative or tranquilizer properties, it is **not** necessary for patients to refrain from operating an automobile or other mechanical equipment while on Tigan medication.

Packages: Capsules, 100 mg, blue and white — bottles of 100 and 500; 250 mg, blue — bottles of 50. Ampuls, 2 cc (100 mg/cc) — boxes of 6 and 25. Multidose vials, 20 cc (100 mg/cc) — boxes of 1. Suppositories, 200 mg — boxes of 6.

References: 1. W. Schallek, G. A. Heise, E. F. Keith and R. E. Bagdon, **J. Pharmacol. & Exper. Therap., 126**:270, 1959. 2. O. C. Brandman, paper read at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959. 3. I. Roseff, W. B. Abrams, J. Kaufman, L. Goldman and A. Bernstein, **J. Newark Beth Israel Hosp. 9**:189, 1958. 4. W. B. Abrams, I. Roseff, J. Kaufman, L. M. Goldman and A. Bernstein, **New York J. Med., 59**:4217, 1959. 5. O. Brandman, **Gastroenterology, 38**:777, 1960.

TIGAN® Hydrochloride — 4-(2-dimethylaminoethoxy)-N-(3,4,5-trimethoxybenzoyl) benzylamine hydrochloride

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Editorial

The physician and *The United States Pharmacopeia*

Largely through lack of publicity, the medical profession and the general public have little knowledge of the importance of *The United States Pharmacopeia* in modern medicine. Some physicians consider the book of interest only to pharmacists. Some pharmacists believe that the book is of more importance to governmental agencies such as the Food and Drug Administration than it is to them. The fact is that the USP from its inception has been primarily a list of the therapeutically most effective agents at the time of its publication. Of necessity, technical standards for these agents are required. Thus, because of these standards, pharmacists, manufacturers, and the government find the USP of great value, although initially it was primarily for the guidance of physicians in the choice of drugs.

The first edition was made possible chiefly through the efforts of Dr. Lyman Spalding of New York when he proposed on Jan. 6, 1817, to the New York County Medical Society:

1. "That a convention should be called in each of the four grand divisions of the United States, to be composed of delegates from all the medical societies and schools.
2. "That each district convention should form a Pharmacopeia, and elect delegates to meet in general convention in the city of Washington, on the first of January, 1820.
3. "That the general convention should, from the district Pharmacopeias, form the national work."

In January, 1820, Dr. Spalding and ten other physicians met as delegates to the first United States Pharmacopeial Convention at Washington, D. C., in the old Senate Chamber now used by the Ways and Means Committee. A painting by Robert Thom depicting this historic occasion now hangs in the Lyman Spalding Library of the Pharmacopeia Building in New York City.

The object of the USP is stated in the preface of the 1820 edition as follows:

"It is the object of a Pharmacopeia to select from among substances which possess medicinal power, those, the utility of which is most fully established and best understood; and to form from them preparations and compositions, in which their

powers may be exerted to the greatest advantage. It should likewise distinguish those articles by convenient and definite names, such as may prevent trouble or uncertainty in the intercourse of physicians and apothecaries.

"The value of a Pharmacopeia depends upon the fidelity with which it conforms to the best state of medical knowledge of the day. Its usefulness depends upon the sanction it receives from the medical community and the public; and the extent to which it governs the language and practice of those for whose use it is intended."

This multiple objective has been adhered to by each successive Committee of Revision, and the same objective, quoted from the first edition, appears in the preface of USP VXI, published in 1960.

The first two decennial revisions were entirely in the hands of physicians. It was not until 1850 that pharmacists helped actively in the revision, that leading to USP III, and not until 1880 was emphasis placed on developing standards and test methods. The new chairman of the Committee of Revision, Charles Rice, recognized that definitions were meaningless without them. In 1910, the Committee of Revision was enlarged to fifty members and included seventeen physicians and thirty-three pharmacists "or other technical experts." In 1940, analytic chemists were brought into the revision program in a more formal way and since then have assumed an increasingly important role in helping to establish standards for each drug selected.

The USP is maintained and revised quite independently of the United States Government. Indeed, the Federal government seems to have been almost oblivious to the need for a pharmacopeia and drug standards generally until Congress passed the Pure Food and Drugs Act of 1906. The latter recognized the USP as an "official compendium," thereby giving definite status to the drugs and the standards provided in the USP. The Food and Drug Administration which ultimately came into being under the terms of the Act was em-

powered to enforce the USP standards by authority to seize any interstate shipment of an article that failed to meet those standards set up for it. This authority was retained and extended in 1938 when the law was brought up to date in the form of the Federal Food, Drug and Cosmetic Act. A companion volume, *The National Formulary,* published by the American Pharmaceutical Association in Washington, D. C., lists drugs not included in *The United States Pharmacopeia* but which nevertheless are used. Standards are provided for those drugs, and these standards also are official under the Food, Drug, and Cosmetic Act.

In accordance with the plan conceived by Dr. Spalding in 1820, the Pharmacopeial Convention still meets only once every 10 years. Its make-up has changed little, and it now includes representatives from medical colleges and societies, pharmacy schools and societies of pharmacists, the pharmaceutical industry, and a number of allied scientific societies. The number of organizations entitled to appoint delegates to the 1960 Convention in Washington, D. C., was 277. An important function of the Convention is the selection of qualified experts to conduct the revision program. These constitute the sixty man General Committee of Revision which now includes twenty physicians and forty experts from pharmacy and related sciences. Prior to the 1960 Convention, candidates for election to the Committee of Revision were carefully selected to provide two nominees for each position. The qualifications of each candidate were listed in a booklet made available to each delegate. Nominations also were received from the floor. The Convention then elected the Committee of Revision, which is responsible during the 1960-1970 decade for two major revisions of the USP and such supplements as may be needed. A new Board of Trustees also was elected for the 10 year period.

The actual job, then, of selecting drugs and establishing standards is in the hands of the Committee of Revision.

The USP is indeed fortunate in having as its director Dr. Lloyd C. Miller, who is particularly well qualified for the post since he has previously been associated with the Food and Drug Administration and also has worked as a pharmacologist in the laboratories in one of the larger drug companies. He is responsible for publication of each edition of the USP. This means that he must work very closely with all subcommittees and with the Board of Trustees. He must also be cognizant of developments in the manufacturing field in therapeutics and in the Food and Drug Administration program.

It cannot be stressed too strongly that although standards are given for each drug in the USP, it is not just a book of standards. The most important task is that of selection of those drugs of established merit which are intended to reflect the best teaching and practice of medicine, and this is the job of the USP Subcommittee on Scope. This committee consists of twenty-one physicians and pharmacologists, representing the major specialities of medicine, aided by pharmacists.

The physicians are responsible for those aspects of the selection process that involve therapeutic merit of drugs, and the pharmacists are concerned with the pharmaceutic forms of the drugs as well as aids to compounding.

The selection may be neither capricious nor arbitrary. Each physician member of the Subcommittee on Scope heads a panel of experts who advise on the selection of drugs. Information from many sources on each individual drug and groups of drugs having similar pharmacologic actions is made available to the committee and panel members. The final selection of a drug for the USP therefore carries with it the considered judgment of a group of medical experts, so that only the best and most effective therapeutic agents are chosen. It is only after such a selection that the analytic and pharmaceutic chemists who serve on other USP subcommittees can proceed to define standards for the drugs. Thus, far

from being just a book of standards as sometimes stated, the USP is a blue ribbon list of the most effective therapeutic agents available at the time the selection is made.

The physician members of the Subcommittee on Scope serve also on another USP committee, which describes the category in which each drug belongs and defines the usual dose. The fact that the USP has official status under the Food and Drug Act makes it difficult for the Committee of Revision to go beyond that, even though this is often urged.

Combinations of drugs generally have been avoided, except in the case of vitamins. Also, in view of the adverse reports of independent investigators on the reliability of absorption of the "delayed action" dosage forms, these have been omitted from USP XVI.

The problem of drug nomenclature concerned the physicians who drafted the 1820 USP, and it has been of increasing concern to each succeeding Committee of Revision which has had the obligation to choose the official name for a drug. At present, however, by the time a drug is up for USP consideration, it has already been approved for use by the Food and Drug Administration. The drug has become familiar under a short, easily pronounced brand name which cannot be appropriated as the USP title without the owner's consent, and this is rarely forthcoming. A nonproprietary, or generic, name has generally had the approval of the Council on Drugs of the American Medical Association. Generic names are often long and almost unpronounceable, as, for example, diethylcarbamazine citrate and prochlorperazine ethanedisulfonate. Discussions are now under way which may change this system in the future so that new drug names will be shorter and easier to pronounce and to remember.

The USP organization serves in many ways in addition to publishing the USP. One of the best known of these services is to make available at a nominal charge to

manufacturers and others USP Reference Standards for use as comparison standards in the assaying and testing of many of the USP drugs. These reference standards are used the world over.

In view of the official status accorded by federal agencies to the standards set by the USP, there has been considerable pressure to have USP standards set up for biologic testing materials and for devices used in surgical procedures. Standards are already made available in the USP for such surgical items as sutures and purified (absorbent) cotton. Up for consideration by the new Subcommittee on Scope are such items as materials used in connection with bone surgery, for instance, metallic pins and plates. Also to be considered are special prostheses used in vascular surgery.

The future of *The United States Pharmacopeia* depends on the support which it receives from the medical profession, hospital formularly committees, and pharmacists. It is hoped that the USP can maintain its independent, nonprofit, scientific status so that it may continue to call upon those who voluntarily devote their time and energy to selecting the best therapeutic agents and providing standards for them.

Important to the future of the USP is whether its role in standards making will be taken over further by the Federal government. Bills are now before the Congress to extend the certification of antibiotics. If enacted, this legislation will be a long step toward certification of all drugs. Under the form of control to which most antibiotics are subject, the Food and Drug Administration not only enforces the standards but it creates them, all at the expense of the antibiotic producers. It scarcely needs to be pointed out how great are the pressures thereby developed on the personal integrity both of those charged with administering the program and of those who are in virtual bondage to the regulating agency.

The record simply does not justify the present degree of antibiotic certification, much less its extension. Further, the procedure violates the long-established principle of placing responsibility for drug standards in the hands of those who respectively prescribe and dispense the drugs themselves, i.e., physicians and pharmacists. If any change in the present law is made short of taking the antibiotics off certification entirely, it should be to convert the system to that which prevails for the insulins. These are all subject to presale certification by the Food and Drug Administration, which is empowered to establish standards only when the USP (or the *National Formulary*) has not yet done so, and in no case may the standards differ from those set up by either of the two compendia. This system, which has operated most effectively since 1940, should have been adopted for the antibiotics when, as all agreed at the time, wartime conditions called for some sort of federal control over their potency and quality.

The USP listing will continue to be a mark of merit for every drug so recognized. Under way at the present time are plans to speed up the process of evaluation of new drugs so the USP list will be kept constantly up to date. The aim is to recognize or reject the new drugs with such promptness that physicians will be able to practice the best possible medicine with only USP drugs.

Arthur C. DeGraff, M.D.

Addiction liability of an isoquinoline analgesic, 1-(p-chlorophenethyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline

The addictiveness of a new synthetic isoquinoline analgesic (I-K-1) has been compared with that of morphine, codeine, and D-propoxyphene in former opiate addicts. Single oral doses of 600 and 1,200 mg. of I-K-1 (ten to seventeen times the recommended analgesic dose) did not induce subjective or objective patterns of morphinelike effects. Intramuscularly and intravenously, I-K-1 was identified as an opiate, but it was not possible to give repeated doses of the drug by these routes because of its water insolubility and tissue irritant properties. When I-K-1 was substituted for morphine in patients addicted to morphine, it partially prevented development of withdrawal symptoms, but it was only one-seventh as potent as codeine in this respect.

In a direct addiction test that lasted 60 days, using maximally tolerated doses (750 to 1,500 mg. orally daily), I-K-1 was disliked by former addicts and when it was discontinued abruptly withdrawal signs were insignificant. It is concluded that I-K-1 has substantially less addiction liability than morphine and codeine and even less addictiveness than D-propoxyphene.

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An isoquinoline derivative, 1-(*p*-chlorophenethyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (I-K-1)* (Fig. 1), represents a new class of synthetic analgesics. Brossi and associates[3] reported that in the mouse, rat, and rabbit I-K-1 had an analgesic potency comparable to that of codeine and, in these species, its over-all pattern of effects was similar to that of morphine. In the cat, it induced less excitation than morphine. Deneau and Seevers* found that the ability of I-K-1 to suppress signs of abstinence from morphine in addicted monkeys was very low.

Keats and Telford† evaluated the analgesic potency of I-K-1 in 96 postoperative
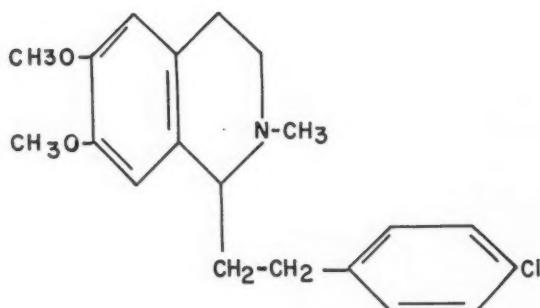
Fig. 1. Structural formula of 1-(p-chlorophen-ethyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline.

patients, using doses of 5, 15, 30, 50, and 75 mg. per 70 Kg. intramuscularly and compared these effects with 10 mg. of morphine per 70 Kg. In addition, 15 and 30 mg. of I-K-1 per 70 Kg. intramuscularly was compared directly with 50 mg. of codeine sulfate in an experiment with 30 postoperative patients. From these experiments, Keats and Telford concluded that intramuscular I-K-1 was approximately twice as potent as codeine as an analgesic. They also gave 30 preoperative patients 50 mg. of I-K-1 per 70 Kg. intramuscularly to determine its subjective effects. These effects, like those after a placebo, were not morphinelike and were significant only in that one-half of the patients complained of burning and soreness at the injection site.

Sadove, Ali, and Schriffrin[12] compared analgesia after 20 and 40 mg. doses of I-K-1, 30 mg. of codeine, and a placebo, all given orally in double blind fashion to 40 postoperative patients on an orthopedic service. In another study Sadove, Schriffrin, and Ali,[13] using 43 male surgical orthopedic patients, compared fifty oral doses of each of the following agents: 32 and 65 mg. of D-propoxyphene hydrochloride, 30 and 60 mg. of codeine sulfate, 30 and 60 mg. of I-K-1, and a placebo. These authors concluded that 65 mg. of D-propoxyphene, 60 mg. of codeine, and 60 mg. of I-K-1 caused equal analgesic effects. In addition, the latter authors treated 10 patients who had chronic pain and who could not tolerate codeine, with 60 mg. oral doses of I-K-1

(two to six times daily) for 6 weeks to 4 months with beneficial effects.

Lasagna* compared oral doses of 60 mg. of I-K-1, 60 mg. of codeine, 60 mg. of D-propoxyphene, and 600 mg. of acetylsalicylic acid with placebos in 53, 53, 39, and 48 patients, respectively, for relief of postpartum pain. In these trials, I-K-1 and D-propoxyphene were not better than a placebo, but codeine and aspirin were more effective than placebos.

Foldes† administered I-K-1 to 10 patients by intravenous infusion as a supplement to thiopental and nitrous oxide anesthesia. It was as effective as meperidine for this purpose, and adverse respiratory effects were definitely less than those after comparable analgesic doses of alphaprodine and probably less than those after meperidine.

Studies at the Addiction Research Center indicate that I-K-1 has less addiction liability than codeine and D-propoxyphene. Therefore, in view of the possible favorable dissociation of addictiveness and analgesia, studies of the addiction liability of I-K-1 relative to those of codeine, D-propoxyphene, and morphine will be presented in detail.

Methods

The subjects utilized in these studies were physically healthy adult Negro or white men between the ages of 26 and 40 who volunteered for the experiments while serving sentences for violation of federal narcotic laws. All were former opiate addicts with character personality disorders.

I-K-1, codeine phosphate, D-propoxyphene hydrochloride, and a starch placebo were administered orally in identically appearing capsules, except in one experiment in which a solution of I-K-1 in dilute hydrochloride (pH 3) was given. For intramuscular and intravenous injection, sterile aqueous solutions of morphine sulfate, codeine phosphate, and D-propoxyphene hydrochloride were prepared, but

it was necessary to add 0.1N hydrochloride acid to a pH of 2.9 to 3 to dissolve I-K-1 and maintain it in solution.

Except when specified, each patient was used as his own control in crossover experiments which were either double blind (both subjects and observers were unaware of the nature and amount of medication) or single blind (subjects only did not know the nature and schedule).

Statistical comparisons between drugs, when the design permitted, were made by the paired t test,[4] and relative potencies were determined using the method described by Bliss[2] or the method of Houde and Wallenstein.* For convenience of presentation, the specific details of methods and results will be described for each experiment.

Effects

Experiment 1. Comparative effects of single oral doses of I-K-,1 D-propoxyphene, and a placebo.

Methods. In these studies in nontolerant former opiate addicts, I-K-1 was compared with other drugs and a placebo employing the single dose opiate questionnaires,[8] which give ratings of patients (subjective) and observers (objective). Questionnaires were completed 1, 2, 3, 4, 5, and 7 hours after medication. The observers also measured pupillary diameter at the same intervals.[10] Statistical comparisons between drugs were based on total response scores (TRS)[8] and were obtained for each parameter by adding the scores or responses recorded at the six intervals and evaluating these totals by the paired t test.[4]

Results. Preliminary experiments with several subjects indicated that doses of less than 600 mg. (total) of I-K-1 induced no significant effects. A crossover single blind study was then set up using 11 nontolerant former morphine addicts. Each received in randomized order I-K-1 (600 and 1,000 mg.), D-propoxyphene (300 and 600

mg.), and a placebo at weekly intervals. Even though the doses of I-K-1 were high, patients had difficulty in distinguishing it from a placebo, whereas they readily identified 300 and 600 mg. of D-propoxyphene as active drugs with many opiatelike characteristics. Of the parameters measured—"feel drugs," identification as "dope," opiate symptoms, and degree of "liking"—I-K-1 was only significantly different from a placebo for one parameter, the opiate symptoms score on the 1,000 mg. dose. On the other hand, D-propoxyphene was significantly different ($p < 0.05$) from a placebo in all parameters in a 600 mg. dose and different from a placebo, except for the measure identification as "dope," in the 300 mg. dose. Furthermore, whereas D-propoxyphene constricted the pupils in proportion to the dose, neither dose of I-K-1 caused constriction of the pupils.

The experiment mentioned above was carried out with I-K-1 as the base. Brossi and associates[3] used the hydrochloride in studying the drug in animals. Since the hydrochloride might be absorbed more rapidly, another experiment was performed with a solution of I-K-1 hydrochloride at pH 3 orally. A single dose of this solution containing 300 mg. of I-K-1 was given to 7 subjects and a dose of 600 mg. to 7 other nontolerant subjects; observations were carried out as described above, except that the study was single blind. None of the subjects identified either dose as "dope." Furthermore, the scores on opiate symptoms and "liking" were very low. Slight miosis followed the 600 mg. dose. The average maximum pupillary constriction in these 7 subjects was 0.9 mm. In contrast, 11 subjects who received 600 mg. of D-propoxyphene showed an average maximum pupillary constriction of 2.2 mm.

Experiment 2. Effects of single doses of I-K-1 intramuscularly.

Methods. This was evaluated in twenty-eight tests (single blind) on 15 subjects, utilizing a dose range of 8 to 400 mg. and single dose opiate questionnaires and observations as outlined in experiment 1.

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*The method of R. W. Houde and S. L. Wallenstein, as cited by Beecher.[1]

Results. Subjective effects were first obtained with a dose of 125 mg. With a dose of 400 mg., 5 of 6 patients identified I-K-1 as "dope" and the pupils were constricted moderately. With all the higher doses (200 to 400 mg.), patients complained of pain at the injection sites and consistently developed inflamed and indurated areas which subsided slowly but progressively over several days.

Experiment 3. Comparative effects of single intravenous doses of I-K-1, D-propoxyphene, codeine, and a placebo in nontolerant subjects.

Methods. In a crossover single-blind study, using the methods of experiment 1, 16 nontolerant subjects each received intravenously at weekly intervals, in randomized order, I-K-1 (60 and 120 mg.), D-propoxyphene (120 mg.), codeine phosphate (60 and 120 mg.), and a saline placebo.

Results. In corresponding dosages, I-K-1, codeine, and D-propoxyphene induced morphinelike effects of comparable magnitude as demonstrated by the ratings of the patients and those of observers. Furthermore, corresponding doses induced comparable pupillary constriction in the case of all drugs except the placebo. The relative potencies of codeine and I-K-1 follow: (1) using the total response scores of opiate symptoms, 1 mg. of codeine equals 1.05 mg. of I-K-1 with 95 per cent confidence limits of 0.97 to 1.14 mg., and (2) using the decreases in pupillary diameter, 1 mg. of codeine equals 0.83 (0.67 to 1.03) mg. of I-K-1.[2]

Experiment 4. Comparative effects of single intravenous doses of I-K-1, D-propoxyphene, and morphine in partially tolerant subjects.

Methods. In 8 patients who had been addicted to 240 mg. of morphine sulfate daily for 310 to 315 days, the morphine was withdrawn and methadone was substituted. The dosage of the latter was gradually reduced over a period of 10 days. During the next 7 to 10 days, none of the patients received any medication. At this time they were in good physical condition but were still manifesting varying degrees of lethargy, anorexia, and insomnia, and all were repeatedly requesting a "pickup" (a potent opiate). (Under usual circumstances, an unstable physiologic and psychologic state persists for approximately 2 months after withdrawal of opiates, and under these conditions such patients are more likely to seek opiatelike drugs. Therefore, evaluation of drugs in such patients was thought to be of interest.) At this time, using a single blind, randomized, crossover design, each subject received at weekly intervals a single intravenous dose of I-K-1 (180 mg.), D-propoxyphene (180 mg.), and morphine sulfate (25 mg.). Observations were carried out as outlined in the preceding experiments.

Results. The parameters tabulated—identification as "dope," opiate symptoms score, "liking" score, and decrease in pupillary diameter—are as shown in Fig. 2. It is apparent that I-K-1, when given intravenously, has morphinelike characteristics of limited degree, but in this study, when partially tolerant subjects were used, these effects were less than those of D-propoxyphene except for pupillary constriction and distinctly less than those of morphine.

Suppression of abstinence reaction

Experiment 5. Suppression of abstinence reaction from morphine with I-K-1 orally.

Methods. This was evaluated in a single blind test using 9 subjects stabilized on 280 mg. of morphine daily, and the results obtained were compared with those observed when a placebo was substituted for 24 hours in the same subjects.[6] Observations were made at hourly intervals from the eleventh through the twenty-fourth hours.[6, 9] Four of these subjects received four doses of I-K-1 (300 mg. each) 6, 10, 16, and 20 hours after the last dose of morphine, while 5 subjects were given five 300 mg. doses during the 24 hours when I-K-1 was substituted.

Results. For all 9 patients, the mean total area scores and standard deviations

IDENTIFIED AS "DOPE"

OPIATE SYMPTOMS

"LIKING SCORE"

PUPILLARY DECREASE

AVERAGE RESPONSES OR SCORES OF PATIENTS

HOURS AFTER I.V. INJECTION
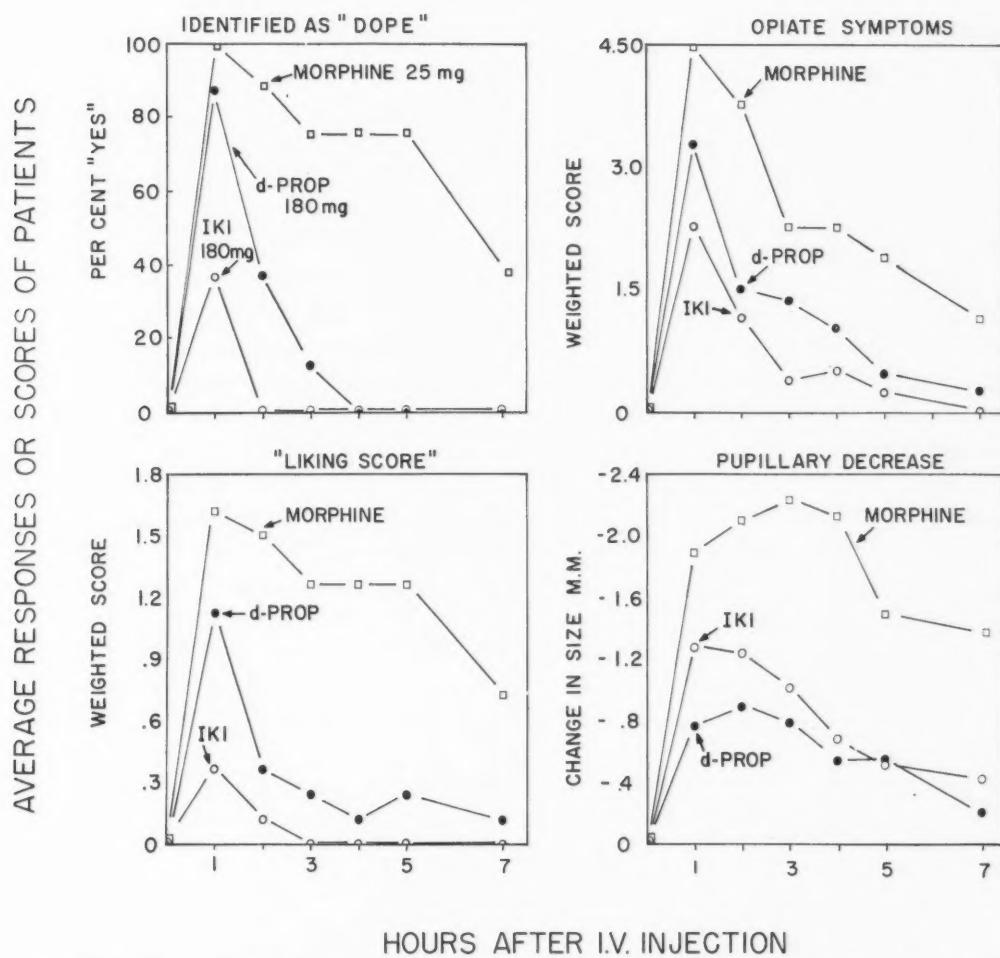
Fig. 2. Average effects of morphine (25 mg.), D-propoxyphene (180 mg.), and I-K-1 (180 mg.) administered intravenously to 8 partially tolerant opiate addicts.

(summation of hourly points of the time-action curves for the first 10 hours plus one-half the hourly points for the eleventh hour) were 126.2 ± 17.3 when I-K-1 was substituted and 163.9 ± 18.9 when a placebo was substituted, a difference which is significant by the paired t test. Hence, orally administered I-K-1 partially suppresses the reaction of abstinence from morphine.

Experiment 6. Suppression of abstinence reaction from morphine with intramuscular I-K-1.

Methods. Using 9 subjects stabilized on 240 mg. of morphine daily, three levels of I-K-1 (300, 600, and 1,200 mg.) and two levels of codeine phosphate (100 and 200 mg.) were compared in a randomized sequence on a double blind crossover basis

during 24 hour substitutions, and observations were made as in experiment 5.[6, 9]

Results. I-K-1 partially but significantly suppressed symptoms of abstinence from morphine by the paired t test (Fig. 3). However, it was only one-seventh as effective as codeine in this respect, since 1 mg. of codeine was found to be equivalent to 6.9 (1.17 to 11.53) mg. of I-K-1 (statistical procedure of Houde and Wallenstein).* Marked areas of inflammation and induration surrounded the sites of injection of I-K-1, and with the higher doses (600 and 1,200 mg. daily), the inflammation was so severe that treatment with wet packs and electric pads was required for about a week.

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*As cited by Beecher.[1]
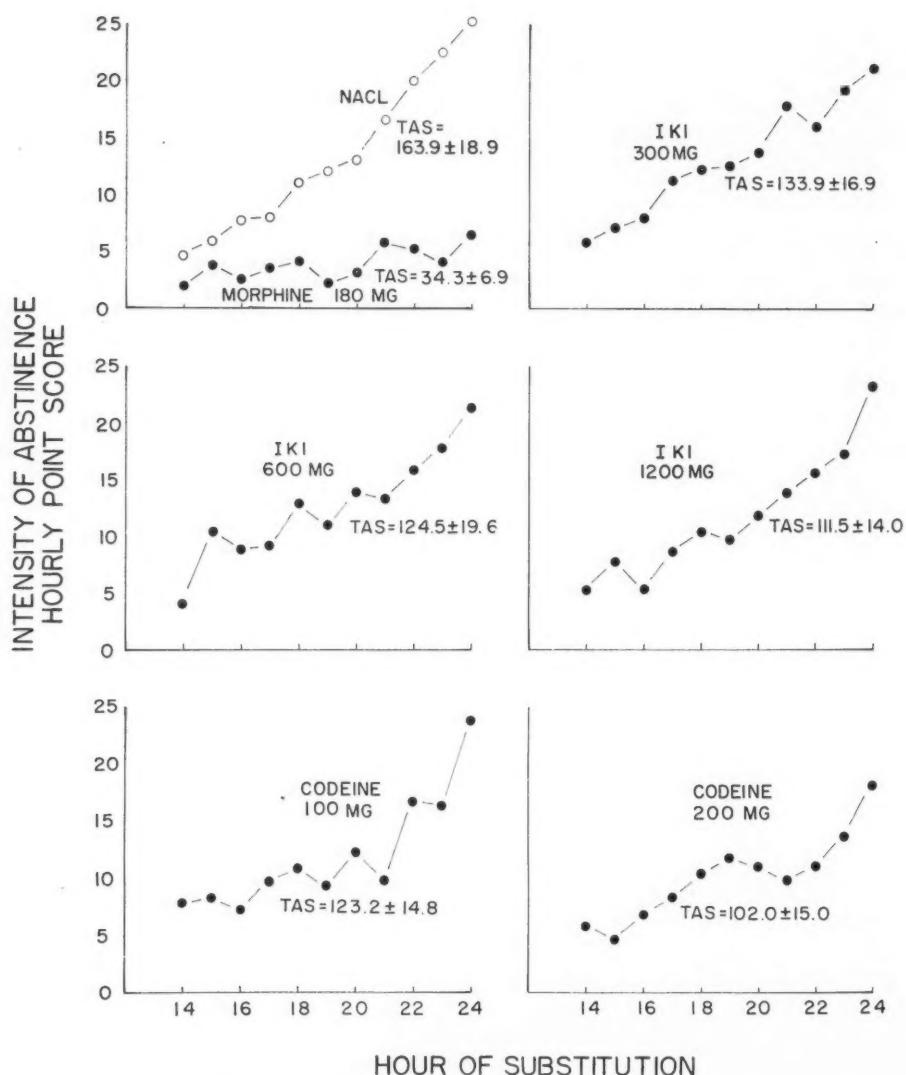
Fig. 3. Results of 24 hour substitution tests to suppress the abstinence reaction to morphine. The same subjects were compared for intensity of abstinence reaction (hourly point scores) when the following agents, administered intramuscularly, were substituted for 240 mg. of morphine daily: saline, I-K-1 (300, 600, and 1,200 mg.), codeine (100 and 200 mg.), and morphine (180 mg.). Then dosage was resumed at a rate of 240 mg. of morphine daily. Each point represents the average intensity of abstinence reaction observed for 9 subjects. (TAS) Scores refer to the total area under the curve and standard error of the mean for eleven observations.

Experiment 7. Suppression of abstinence reaction from morphine with intravenous I-K-1.

Methods. Using 10 subjects dependent on 240 mg. of morphine sulfate daily, two daily levels of I-K-1 (180 and 360 mg.) were compared with two similar dosages of codeine phosphate and D-propoxyphene and with 9 and 18 mg. of morphine sulfate and a placebo. Drugs were administered in a randomized sequence on a double blind basis during 24 hour substitutions and observations made as in experiment 5.[6, 9]

Results. Three patients received 360 mg. of I-K-1 daily and 2 patients 180 mg. daily. At this time, the study was terminated because of pain and inflammation and partial occlusion of the veins at the site of injection. Because of the small number of sub-

jects, only a qualitative interpretation of the data is possible, namely, that abstinence reaction was partially suppressed by intravenous I-K-1.

Six of 10 patients received both doses of codeine intravenously during the substitution, and the abstinence reaction was partially suppressed. However, it was necessary to terminate the experiment because 1 patient had a mild and another a severe vascular collapse while codeine was being substituted at a rate of 360 mg. (divided among three equal doses) for 240 mg. of morphine daily. The mild reaction came after the first dose of codeine and the severe one after 95 mg. of the third 120 mg. dose had been injected. In the latter case, the patient requested the injection be stopped. There was extensive erythema of the skin, followed by weakness and excessive sweating. It was not possible to record the blood pressure for about 8 minutes, a severe headache developed which lasted for 24 hours, and edema of the skin persisted for 48 hours.

All 10 patients received 360 mg. (divided among three equal doses) of D-propoxyphene intravenously, but only 9 were given the 180 mg. dose since 1 patient insisted that he not be given any more, complaining "it was like LSD [lysergic acid diethylamide], the people in the painting on the wall became alive, I was very nervous, and I couldn't sleep for several nights." D-Propoxyphene partially suppressed abstinence reaction from morphine as compared to a placebo, however it was not possible to augment the degree of suppression by increasing the dose of D-propoxyphene. In fact, the mean total abstinence scores (area under the time-action curve and the standard error) for the eleven observations were the same for the two dose levels (132.7 ± 6.46 for the 180 mg. dose and 131.4 ± 8.43 for the 360 mg. dose). For comparative purposes, when a placebo was substituted for morphine in the same patients, the total mean abstinence score was 179.5 ± 12.65; when morphine was continued (positive control), this score was only 42.85 ± 7.43.

Addiction

Experiment 8. Direct addiction to oral I-K-1.

Methods. This was conducted on a double blind basis in 5 subjects. The addictiveness of I-K-1 was compared to that of a placebo, the agents being administered in identically appearing capsules. Each patient was observed for 10 days while receiving placebos, and then I-K-1 was given in increasing dosages for 18 days, after which it was discontinued on a double blind basis for 2 days. Then, medication was resumed for an additional 42 days, medication was again discontinued abruptly by substituting identically appearing placebo capsules, and patients were observed for symptoms of abstinence for 10 days. The precautions taken and observations made in all parts of the experiment were those described by Kolb and Himmelsbach[11] and by Himmelsbach.[9] In addition, during the entire study the chronic dosage questionnaires (patients' and observers' ratings) were completed daily at 7 P.M.[8] The effects of I-K-1 on respiratory rate and blood pressure in this study were compared with those of codeine[7] and of D-propoxyphene[5] in other experiments using different subjects. In addition, the results obtained from tabulating the chronic dosage opiate questionnaires, independently completed by patients and aides while I-K-1 was being administered in this test, were compared with those obtained for codeine and a placebo administered on an increasing dosage schedule in another, similar experiment.[8] Complete blood counts and urinalyses were made every 2 weeks.

Results. None of the 5 patients liked the effects of I-K-1; they complained of nausea, gas on the stomach, nervousness, headache, loss of appetite, and sluggishness or sleepiness. None identified the drug as resembling any which they had previously received. One patient, however, quite consistently stated that the medication was "dope," but he complained repeatedly about the effects of the drug and said he

would not take it if he were not on a test. In all subjects, after 18 days of medication I-K-1 was discontinued for 2 days on a double blind basis. During this interval no significant signs of abstinence reaction developed; however, 2 of the 5 patients reported subjectively that they were having mild withdrawal symptoms. When medication was resumed after 48 hours of abstinence, all patients began to complain of toxic effects as noted above during the first 18 days of medication and 1 quit the test after 22 days because of disturbing gastrointestinal effects. Only 1 of the remaining 4 patients continued taking the drug at the planned daily dose level of 1,500 mg. In 2 patients it was necessary to reduce the dosage to 75 per cent of the maximum attained and in 1 patient to 50 per cent, or 750 mg. daily, because he was tremulous, nervous, nauseated, and losing weight. It was the latter patient who

quite frequently identified I-K-1 as "dope" (opiate). When the physician in charge reduced the dosage, every patient was relieved. Toxic symptoms declined progressively after the dosage was reduced. At no time did any patient ask to have the dose of I-K-1 increased, whereas in practically every instance when former addicts chronically receive a drug with opiatelike effects, they repeatedly request that the dosage be augmented.

A comparison of I-K-1 chronically administered in this experiment with codeine and a placebo given on a double blind basis to another group of 8 patients in a similar experiment is shown in Fig. 4. The small proportion of responses that indicated I-K-1 was "dope" is noteworthy; none reported they would like to take I-K-1 daily. On the contrary, 50 per cent stated they felt so badly they would stop taking the drug if they were not on a test (Fig.
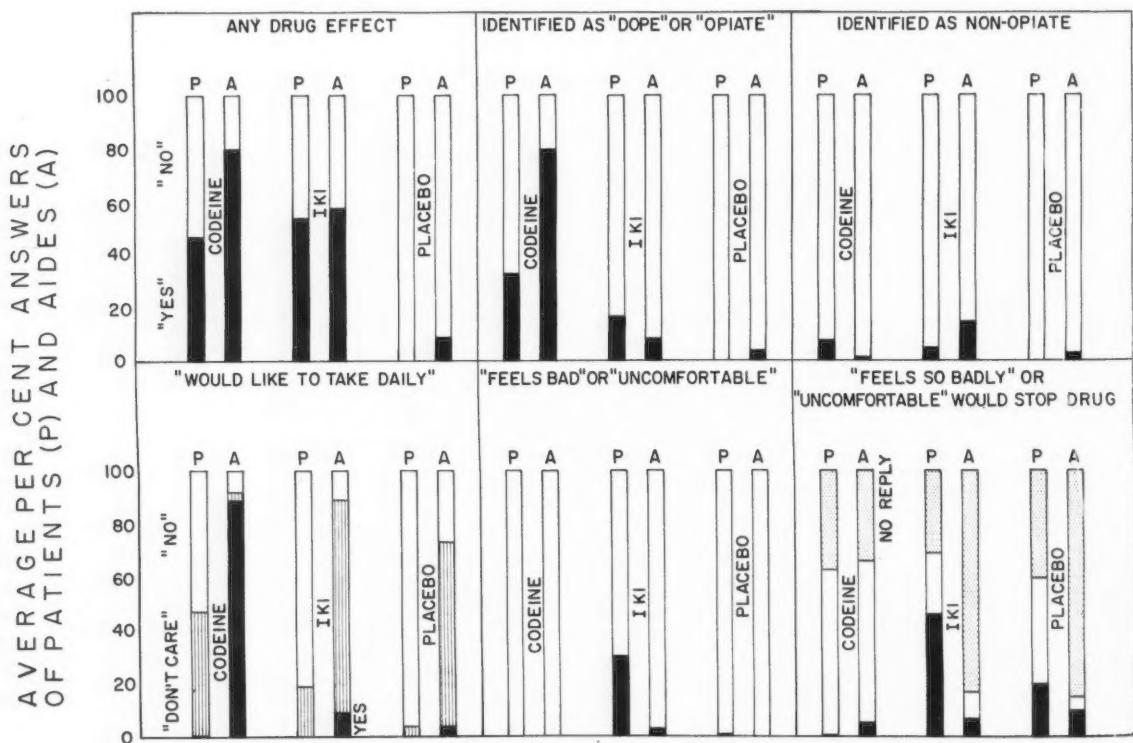
Fig. 4. Oral direct addiction test of I-K-1. Results are summarized of the chronic dosage opiate questionnaires independently completed by patients (P) and aides (A). These compare responses obtained in the case of addiction to I-K-1 for 60 days with those obtained for codeine and a placebo. The latter agents were administered for 18 to 20 days in randomized fashion in another experiment.

4). On the other hand, codeine provoked no such undesirable effects even though the dosage attained was slightly higher than that of I-K-1 (Fig. 4, Table I).

Fig. 5 shows the average intensity of abstinence reaction which developed after 60 days of receiving I-K-1 as compared with 53 days of D-propoxyphene and 60 days of codeine. The maximum intensity of abstinence reaction in the case of I-K-1 averaged only eight points for the 4 subjects who completed the test—an insignificant score. In fact, the average daily point score for these patients during the 10 days of withdrawal was only two points more than the average daily point score observed during the last 7 days while receiving I-K-1. The aides reported that they thought 1 patient exhibited abstinence reactions twice (two positive as compared with thirty-eight negative reports). However, the patients subjectively experienced mild abstinence reactions, since all reported positively one or more times (thirteen positive and twenty-seven negative reports). The degree of abstinence reaction following withdrawal of orally administered I-K-1 is less than that observed after withdrawal of D-propoxyphene and a great deal less than that observed after withdrawal of codeine (Fig. 5).

The effects of I-K-1 (shown in Table I) on respiratory rate and systolic blood pressure are compared with those of D-propoxyphene and codeine, all drugs having been orally administered in different direct addiction tests using different subjects. Codeine significantly depressed both respiratory rate and blood pressure in all patients, and D-propoxyphene significantly depressed respiratory rate in 4 of 5 patients and increased blood pressure in all patients. I-K-1, on the other hand, depressed respiratory rate significantly in only 1 patient and blood pressure was increased significantly in only 1 patient.

Complete blood counts and urinalyses, taken at intervals of 2 weeks, showed no significant abnormalities during chronic administration of I-K-1.
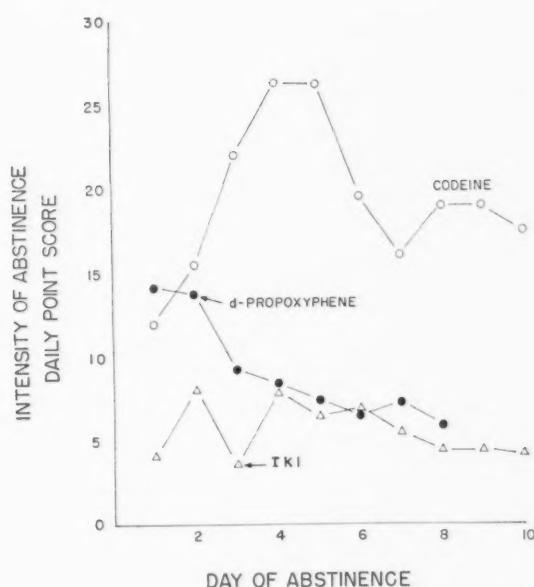
Fig. 5. Abrupt withdrawal of drugs. The average intensity of abstinence reaction after abrupt withdrawal of orally administered I-K-1 is compared with that observed for codeine and D-propoxyphene. The average maximum daily stabilization doses for these drugs were 1,094 mg. I-K-1, 1,375 mg. codeine, and 780 mg. D-propoxyphene.

Experiment 9. Relative addictiveness of I-K-1, D-propoxyphene, codeine, and morphine as determined in a "short" direct addiction test of 7 days using injected drugs.

Methods. In a single blind study, 6 subjects received drugs intravenously and 1 intramuscularly. A "sample" of a high dose of each drug was given at 3 day intervals as follows: 180 mg. I-K-1 or D-propoxyphene was followed by either codeine (120 mg.) or morphine (30 mg.). Each patient rated each of these drugs on the single dose opiate questionnaire.[8] After each subject had received all four single doses, he was asked to review his response to the questionnaire for each sample drug and to rate each on a preference scale ranging from 1 to 4. It was explained to each subject that he would subsequently receive all sample drugs which he elected to take, in randomized order in increasing doses for 7 days; each would be withdrawn for 3 days, then the next drug would be given for 7 days. After evaluating the effects of

Table I. *Comparison of the effects of* D-*propoxyphene, I-K-1, and codeine on respiratory rate and blood pressure*

Days before drug	Days addicted	Patient	Drug	Stabilization dose (mg. per day)	Respiratory rate ± S.E.		Systolic blood pressure ± S.E.	
					Preaddiction	Addiction	Preaddiction	Addiction
9	53	1	D-Propoxy-	600	20.6 ± 0.75	15.8 ± 0.02°	114.8 ± 1.70	124.7 ± 1.10°
		2	phene	825	21.8 ± 0.51	19.4 ± 0.09°	99.9 ± 1.63	105.0 ± 0.79°
		3		825	21.8 ± 0.89	19.4 ± 0.08†	108.3 ± 0.76	119.0 ± 0.31°
		4		825	18.9 ± 0.42	16.8 ± 0.08°	99.9 ± 0.39	107.1 ± 1.08°
		5		825	21.4 ± 0.91	20.4 ± 0.08	107.8 ± 1.12	114.9 ± 0.66°
			Average	780	20.09	18.36	106.14	114.14
10	60	6	I-K-1	1,125	17.6 ± 0.60	15.3 ± 0.21†	114.3 ± 1.32	116.0 ± 0.48
		7		1,500	11.8 ± 0.21	11.7 ± 0.16	116.7 ± 1.93	118.3 ± 0.28
		8		1,125	16.7 ± 0.54	15.6 ± 0.28	109.0 ± 0.93	103.8 ± 0.46
		9		1,125	15.0 ± 0.45	15.2 ± 0.24	114.2 ± 1.35	119.4 ± 0.66°
			Average	1,219	15.28	14.45	113.55	114.37
18	60	10	Codeine	1,200	23.9 ± 0.38	17.4 ± 0.34°	106.4 ± 0.91	97.5 ± 0.60°
		11		1,500	21.2 ± 0.45	18.3 ± 0.21°	112.9 ± 0.83	107.4 ± 0.65°
		12		1,700	16.4 ± 0.51	12.3 ± 0.20°	110.5 ± 1.18	105.5 ± 0.16°
		13		1,100	20.1 ± 0.44	17.0 ± 0.22°	98.5 ± 0.92	97.1 ± 0.62°
			Average	1,375	20.40	16.25	107.07	101.88

°Statistically significant difference as compared with preaddiction p = > 0.01.

†Statistically significant difference as compared with preaddiction p = < 0.05.

Table II. *Seven day direct addiction test of I-K-1,* D-*propoxyphene, codeine, and morphine given intramuscularly and intravenously*

Patient	Route	Days on each drug			
		I-K-1	D-Propoxyphene	Codeine	Morphine
1	Intramuscular	2.3°	6.2°	7.0	7
2	Intravenous	2.5†	4.0°	0‡	7
3	Intravenous and intramuscular	6.3†	7.0	3.5	7
4	Intravenous	0§	4.5°	7.0	7
5	Intravenous	0‖	3.5°	0‖	1.3°
6	Intravenous	0§	5.0°	0‡	7.0
7	Intravenous	0§	7.0	7.0	7.0
	Average		5.3		6.2

°Patient elected to stop taking drug.

†Doctor ordered drug to be stopped.

‡Patient elected not to start drug.

§Doctor ordered drug not to be started.

‖Patient quit test.

the single doses, subjects could elect not to take any of the four sample drugs during the subsequent 7 day phase. Once a subject had started any medication, he could discontinue it any time without penalty and then after a 3 day interval could continue with the next drug. When each patient completed the direct addiction phase, he was again asked to rate each drug in order of preference after reviewing his responses to the chronic dosage opiate questionnaire.[8] Each patient received the same reward whether he elected to take all drugs and continued for all 7 days, elected not to take all, or stopped taking them before the 7 days were up.

The dosage of I-K-1 and D-propoxyphene for the first and second days was 360 mg. administered in three equal doses, and the subsequent daily dosages (divided among four equal doses) through the seventh day were 480, 600, 600, 720, and 720 mg. For

morphine the dosages were 60, 60, 80, 100, 100, 120, and 120 mg., for codeine 240, 240, 320, 400, 400, 480, and 480 mg. daily during the 7 day period. All drugs given intravenously during both phases of the experiment were administered slowly over a 2-minute period. Nalorphine (3 mg.) or a placebo, on a randomized double blind basis, was injected at 9 A.M., 3 hours after the last dose of each drug on the sixth or seventh day of the test.[6] Intensity of abstinence reaction after withdrawal of drugs was measured for 3 successive days.[11]

Results. All 7 patients completed the first phase of the experiment, and their overall preference ratings among drugs was as follows: first choice—morphine by 5 subjects and D-propoxyphene by 2; second choice—D-propoxyphene by 4, codeine by 2, and I-K-1 by 1; third choice—I-K-1 by 4, codeine by 2, and morphine by 1; fourth choice—codeine by 3, I-K-1 by 2, morphine by 1, and D-propoxyphene by 1. Two subjects (2 and 6, Table II) complained that the single injection of codeine provoked such undesirable symptoms that they did not wish to take it subsequently in the direct addiction test. For example, one said, "I have a very severe headache; it isn't 'dope,' it must be 'mother-in-law' medicine."

In the direct addiction phase it was necessary to terminate the study after giving I-K-1 to only 3 subjects because it induced severe phlebitis and venous thrombosis (Table II). All patients complained that morphinelike effects were absent or very weak and that there was pain at the site of injection. The 2 patients who received I-K-1 for 6 days had no symptoms or signs of abstinence reaction when it was discontinued. Since no patient received I-K-1 for 7 days, tests for precipitating abstinence reactions with nalorphine were not completed. At the conclusion of the test, I-K-1 was accorded the preference rating of "nothing" by subjects 1 and 2; subject 3 gave I-K-1 a second choice rating, but this was the only drug which he received intravenously; at no time did he identify I-K-1 as "dope." He persistently stated that I-K-1 produced nausea and made him sick, and by the sixth day of the test, he had marked edema of both arms and hands below the site of injection caused by venous occlusion, so medication was discontinued.

All 7 subjects took D-propoxyphene during the 7-day addiction phase, and even though 5 of the 7 subjects identified D-propoxyphene as "dope," only 2 of them completed 7 days on the drug (Table II). The reasons the patients gave for discontinuing D-propoxyphene included pain at the site of injection, nausea, loss of appetite, dizziness, and nervousness. Patient 2 was so nervous that he was afraid to shave, saying "I might cut myself." Patient 7 took the drug for 7 days intravenously, but his veins became progressively occluded; with the higher doses successive injections had to be given in different veins, and as much as 45 minutes was spent locating a suitable vein. On the other hand, when this patient subsequently received codeine and morphine for 7 days, both drugs could be injected repeatedly into the same vein. Four subjects gave D-propoxyphene a second choice rating, and 3 classified it as "nothing." Neither the nalorphine tests nor abstinence scores showed evidence of physical dependence. Although D-propoxyphene is readily soluble in water and could therefore be injected by addicts, it is doubtful that it would be abused by them either intramuscularly or intravenously for three reasons: (1) as in the case of oral medication, when the dose is so large that adequate opiatelike effects are induced, there are many undesirable effects, particularly nervousness, which may progress to psychosis; (2) if D-propoxyphene is injected rapidly in high dosage, a convulsion may be precipitated (one was observed when an attempt was made to suppress the abstinence reaction from morphine with high doses of D-propoxyphene orally*); and (3) it is a potent tissue irritant.

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*H. F. Fraser and H. Isbell: Unpublished data.

Codeine, when given intravenously, was quite unacceptable to former addict patients. Two of the 6 patients elected not to start the 7 days of addiction, and only 3 of 4 patients who started the test completed 7 days on the drug (Table II). Those taking it intravenously complained that all they felt was a temporary pins and needles sensation. Of the 4 patients who took the drug, 3 gave it a rating of "nothing," and patient 7 rated it the lowest of the three drugs he had taken (Table II). Among the 3 patients who were on the drug for 7 days, the nalorphine score was greater than that after a placebo in 2 of 3 patients, and the intensity of abstinence reaction was mild after withdrawal.

Morphine was definitely the drug of choice and the most addictive drug in the series. Six of the 7 patients gave it first preference rating and completed the 7 days of medication (Table II). One subject (patient 5, Table II), however, stopped taking morphine after the first injection on the second day because of repeated vomiting. He also decided not to take any more of the drugs scheduled for this test. In the other subjects who completed the study, nalorphine scores exceeded those for saline in 5 of 6 cases. When morphine was withdrawn, all patients developed symptoms and signs of abstinence reaction and daily scores exceeded ten points on the second day of withdrawal, except in 1 patient.

Discussion

By every criterion customarily used—degree and quality of morphinelike subjective effects, ability to suppress symptoms of abstinence from morphine, and severity of abstinence reaction after prolonged chronic administration—I-K-1 is a compound of very little or no addictiveness. Its addiction liability, if any, is definitely less than that of D-propoxyphene and codeine, and I-K-1 is, of course, far less addictive than morphine. Even though injected I-K-1 causes morphinelike effects in animals and in man, particularly when given intravenously, the relative insolubility of I-K-1 and the irritating properties of the highly acid solutions required to prevent precipitation make it unlikely that addicts would repeatedly inject I-K-1. In addition, during a direct addiction test when I-K-1 was administered orally in high dosage, unpleasant effects occurred; therefore chronic oral misuse is unlikely.

Since it has been reported that I-K-1 is as effective as or more effective than codeine either orally or intramuscularly, it is possible that a considerable dissociation between analgesia and every aspect of addictiveness has been achieved in I-K-1. However, since Lasagna* found I-K-1 ineffective as an analgesic in postpartum pain (as he did D-propoxyphene), it will be necessary to await the results of additional clinical investigations on the effectiveness of I-K-1 as an analgesic before concluding that such a dissociation has actually occurred.

Conclusion

The addictiveness of I-K-1 has been compared with the liability of morphine, codeine, and D-propoxyphene in former opiate addicts.

Single oral doses of 600 and 1,200 mg. of I-K-1 (ten to seventeen times the recommended analgesic dose) did not induce subjective or objective patterns of morphinelike effects in nontolerant former opiate addicts, but 400 mg. intramuscularly was identified as an opiate by 5 of 6 nontolerant subjects. Single intravenous doses of 60 and 120 mg. of I-K-1 produced effects comparable to those of identical doses of codeine and D-propoxyphene in nontolerant subjects, but 180 mg. of I-K-1 was less effective than 180 mg. of D-propoxyphene in partially tolerant opiate addicts.

Oral, intravenous, and intramuscular I-K-1 partially suppressed signs and symptoms of abstinence from morphine. Intramuscularly, I-K-1 was only about one-seventh as potent as codeine in this respect.

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*L. Lasagna: Personal communication.

In a direct addiction test of 60 days, using maximally tolerated doses (750 to 1,500 mg. orally daily), I-K-1 was disliked by former addicts, and when I-K-1 was discontinued abruptly, withdrawal signs were insignificant.

An attempt was made to ascertain the addiction liability of I-K-1 by injection using a short addiction test of 7 days and comparing I-K-1 with morphine, codeine, and D-propoxyphene. Insofar as I-K-1 was concerned, it was necessary to terminate the experiment after the drug had been administered intravenously to 3 patients and to 1 intramuscularly because of the marked inflammation at the site of injection (it required a pH of about 3 to maintain I-K-1 in solution).

1-(*p*-Chlorophenethyl)-2-methyl-6, 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, it is concluded, has substantially less addiction liability than morphine and codeine and even less addictiveness than D-propoxyphene.

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The hypotensive effect of imipramine hydrochloride in patients with cardiovascular disease

The untoward effects of imipramine hydrochloride were studied in a group of 41 patients under 60 years of age who were without demonstrable cardiovascular disease and compared with those in a group of 50 patients with various degrees of generalized or coronary atherosclerosis. Effects on the following were investigated: recumbent and standing blood pressures, pulse, electrocardiograms, blood coagulation (bleeding time, clotting time, prothrombin time), liver function (SGOT, alkaline phosphatase, cephalin flocculation, thymol turbidity), and formed elements in the blood (hemoglobin, white blood cells with differential count, platelets).

Among the patients with cardiovascular disease, 4 developed congestive heart failure during imipramine administration, while severe postural hypotension was observed in 10 of 41 (24.4 per cent). Two developed myocardial infarction during a hypotensive period. Moderate hypotension was present in 7 patients (14 per cent). Few significant untoward effects were encountered in the patients without cardiac disease. Severe postural hypotension was not observed; moderate degrees occurred in only 3 of the 41 patients (7.3 per cent).

It is suggested that extreme caution be practiced in the treatment of depressive states in patients with known cardiovascular disease, especially in the older age group. Imipramine should be given in small doses and discontinued if postural hypotension occurs.

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Imipramine hydrochloride is a newly synthesized psychotherapeutic agent[6, 17] that has been under investigation for the treatment of psychiatric depression since 1954, first in Europe,[4, 8-10] and later in the United States and Canada.[2, 11] This drug is of value in both mild and severe depression, especially of the endogenous variety. Its effectiveness in periodic endogenous depression has been claimed to be as high as 70 to 85 per cent. Imipramine has also been used for the depressive phase of manic-depressive psychosis, involutional melancholia, reactive depression, senile de-

pression, and depressions associated with organic brain lesions, the latter three being less responsive to therapy.

Serious untoward effects of imipramine have been rarely reported. Somatic reactions, such as dizziness, weakness, shakiness, dry mouth, excessive perspiration, constipation, heartburn, and paresthesia, were seldom severe enough to justify discontinuance of the treatment. A few instances of cardiac death (coronary occlusion and congestive heart failure) in older patients have been reported recently, but it was difficult to establish whether death was attributable to the drug or was coincidental. However, hypotensive episodes and orthostatic hypotension in elderly patients were not infrequently observed. Most of the available studies have thus far been confined to patients with various types of depression. In these, observations of untoward or toxic effects were incidental to the anticipated therapeutic regimen.

The object of this investigation was to determine the possible untoward effects of imipramine in patients with various types of cardiovascular disease, particularly in the older age group. With this object in mind, imipramine* was given to a random, mixed group of patients admitted to the medical and psychiatric wards of the Philadelphia General Hospital, including elderly patients with cardiac disease (e.g., coronary artery disease, hypertension, valvular lesions, and borderline decompensation).

Methods and materials

A total of 91 patients was studied; 84 were selected from the wards of the Philadelphia General Hospital. These included 54 patients from the medical wards convalescing from various ailments, 20 of whom presented various depressive states, and 30 patients with various psychiatric disorders from the psychiatric wards. Seven patients with coronary disease were studied and treated in office practice. No acutely ill patients were used in this study. Ap-

proximately one-half of the subjects were followed on an outpatient basis after discharge from the hospital.

The patients were divided into two main groups:

Group 1 (patients with cardiovascular disease) consisted of 41 patients over 60 years of age (range 60 to 86, with an average of 69.5 years), all of whom had some form of atherosclerosis, either generalized involvement (28 cases) or arteriosclerotic cardiovascular disease with coronary sclerosis (13), as the outstanding finding. Included in the 41 patients were 10 with diabetes mellitus and 9 with hypertension. Other findings were cor pulmonale (1 case), anemia (4), and pulmonary emphysema (5). Two patients were in mild congestive heart failure at the onset of the study, and 2 developed congestive heart failure during imipramine administration. In addition, 9 patients under 60

Table I. *Clinical diagnoses: Group 1. Patients with cardiovascular disorders*

Diagnosis	Patients		
	Over 60	*Under 60*	*Total*
Generalized atherosclerosis	28		28
Arteriosclerotic cardiovascular disease as outstanding finding	13	7	20
Hypertensive cardiovascular disease		1	1
Rheumatic heart disease		1	1
Associated diseases with first two groups			
Hypertension	9		9
Diabetes mellitus	10	4	14
Congestive heart failure			
Before study	2	1	3
During	2	2	4
Pulmonary emphysema	5		5
Cor pulmonale	1		1
Anemia	4		4
Total	41	9	50

*Tofranil.

Table II. *Clinical diagnoses: Group 2.
Patients below 60 years of age and without
evidence of cardiovascular disorders*

Diagnosis	No. of cases
Various forms of depressions, includ- ing manic-depressive states	15
Schizophrenia, paranoid and mixed	9
Involutional melancholia	1
Pulmonary tuberculosis	13
Lung abscess	1
Cerebrospinal syphilis	1
Mild diabetes mellitus	1
Total	41

years of age (range 43 to 58, with an
average age of 54.6 years) who manifested
coronary artery disease (7 cases), hyper-
tensive cardiovascular disease (1), and
rheumatic heart disease (1) were included
in group 1 (Table I).

Group 2, which served as the basis for
comparison, included 41 patients under 60
years of age (range 16 to 57, with an aver-
age age of 39 years) without demonstrable
evidence of cardiovascular disease. Table
II shows the clinical diagnoses in this
group.

The following studies were performed
routinely during a control period of 3 to 7
days: (1) Blood pressure (Riva-Rocci's
method) and pulse rate were recorded
twice daily in the recumbent and in the

Table III. *Effect of imipramine on
depressive states; group 1*

Type of depression	No. of patients	Marked or complete	Slight	None
		Improvement of depressive state		
Periodic depres- sion	2	2		
Schizoid affective	1	1		
Involutional melancholia	1	1		
Reactive	8	4	1	3
Organic and senile	8	4	3	1
Total	20	12	4	4

standing positions. (2) Control electrocar-
diograms with the twelve conventional
leads were obtained. (3) Blood studies
including erythrocyte, leukocyte, and plate-
let counts and bleeding time (Lee-White
method), clotting time (Ivy's method), and
one stage prothrombin time determinations
were made. (4) Liver control studies in
most patients consisted of cephalin floccu-
lation tests and alkaline phosphatase and
serum glutamic oxalic transaminase deter-
minations. (5) In a group of 10 selected
patients, electrolyte studies were per-
formed which included daily chemical
determinations of urinary electrolyte excre-
tions (sodium, potassium, chloride); radio-
active sodium decay curves were also
obtained on 6 of these. (6) In 20 patients
with depressive states, the daily behavior
pattern was observed, and the patients
were interviewed one to two times daily.

Imipramine was administered in doses of
75 to 200 mg. per day. Most patients, how-
ever, received 100 mg. per day in divided
doses, usually 25 mg. four times a day. The
duration of treatment ranged from 4 to 144
days (average 30 days). Some of the
studies were performed daily (blood pres-
sure, pulse, electrolytes). Others were re-
peated at intervals of 2 to 3 weeks (elec-
trocardiograms, liver and blood studies).
Studies in outpatients were obtained at
weekly intervals. Hospitalized patients
were observed daily to determine the pres-
ence of subjective or objective side reac-
tions.

Results

Clinical observations. Twenty patients
with various depressive states (in group 1)
were closely observed and the effect of
imipramine on the depression evaluated.
Of these, 2 had periodic depression, 1 was
schizoid affective, 1 had involutional mel-
ancholia, and 16 had reactive depressions.
In 8 of the latter, the reactive depressions
were associated with chronic brain syn-
drome. Three exhibited suicidal tendencies,
and 2 committed actual suicidal attempts
during the stay in the hospital. Table III

Table IV. *Recumbent blood pressures*

Data	No. of patients	No change	Decrease	Increase
Group 1, patients over 60	33	22	6	4
Group 2, patients under 60	27	18	5	5
Total	60	40	11	9

summarizes the results of treatment with imipramine on the depressive state in these patients. These results follow the pattern which has been previously reported relative to the efficacy of imipramine.[2, 4, 8-11]

Recumbent blood pressure. In 40 of 60 patients in whom intermittent recordings were obtained throughout the study, the recumbent blood pressure remained essentially unaltered during treatment. In 11 patients, a slight fall in the recumbent blood pressure was observed (average of 20 mm. Hg systolic, 10 mm. Hg diastolic). In 5 patients with hypertension, the recumbent blood pressure fell for several days to weeks (20 to 50 mm. Hg systolic, to 40 mm. Hg diastolic) but returned finally to pretreatment levels. Nine patients showed a slight rise (10 to 30 mm. Hg systolic, up to 10 mm. Hg diastolic) in the recumbent blood pressure. There was essentially no difference in the blood pressure response in the recumbent position between groups 1 and 2 (Table IV).

Blood pressures in standing position. Significant findings were encountered when the standing blood pressures were recorded during the period of imipramine administration and compared with the recumbent recordings. Of the 82 patients who were able to stand during the entire test period, 47 showed a decrease in both the systolic and diastolic standing blood pressures. The grade of this postural hypotension was classified as slight, moderate, or severe. We classified as slight postural hypotension all cases with a decrease in diastolic pressure between 5 and 20 mm. Hg on repeated recordings if the patients manifested no symptoms such as dizziness, weakness, or faintness. Moderate postural hypotension included a decrease of the diastolic pressure between 10 and 20 mm. Hg with associated signs and symptoms of transient dizziness, weakness, sweating, and faintness. Severe postural hypotension was considered to be present when the diastolic pressure decrease was greater than 20 mm. Hg (between 20 and 80 mm. Hg, with an average of 40 mm. Hg) and the patient developed pallor, appeared in a moderate grade of shock, had episodes of sudden fainting, and complained of such prolonged severe weakness and dizziness that imipramine had to be discontinued. Table V summarizes the findings in our 82 patients.

Of the total of 82 patients, 47 showed evidence of postural hypotension. This was slight in 27 patients, manifested by only a slight decrease in systolic and diastolic

Table V. *Standing blood pressures*

Data	No. of patients	No change	Decrease	Increase	Hypotension*			Drug discontinued
					Slight	Moderate	Severe	
Group 1, patients over 60	41	9	32	0	15	7	10	10
Group 2, patients under 60	41	24	15	2	12	3	0	0
Total	82	33	47	2	27	10	10	10

*For classification, see text.

pressures. There were no subjective symptoms and no change in the clinical picture. It occurred in about the same number of patients of group 1 (15 of 50) as of group 2 (12 of 41 subjects).

Moderate postural hypotension was seen in 10 of the 82 patients. These patients complained of dizziness, weakness, sweating, and spontaneous faintness and showed a persistent decrease of diastolic pressure between 10 and 20 mm. Hg (2 showed a decrease of as much as 40 mm. Hg). Seven of these patients belonged in group 1 and 3 in group 2. The symptoms were not severe enough to necessitate discontinuance of imipramine, but the dose was decreased in some of the patients. Four patients who were maintained on the initial dose actually became symptom-free after 2 to 3 weeks, despite persistence of the decrease in diastolic pressure, and felt dizzy for only 1 to 2 minutes after standing. The initial dose in these patients was 100 mg. of imipramine daily, except in 1 patient of group 2, who received 200 mg.; he became symptom-free 2 days after the dose had been decreased to 100 mg. The other 2 patients in group 2 with moderate postural hypotension showed a slight decrease in diastolic pressure on standing before the study (both patients had pulmonary tuberculosis) without subjective symptoms. In addition to more marked hypotension, dizziness and faintness developed after imipramine was administered. Similar observations were made in 3 patients out of the 7 with moderate postural hypotension of group 1.

Ten patients of group 1 (none of control group 2) developed such severe postural hypotension that imipramine had to be discontinued. The hypotension usually began 2 to 4 days after treatment was instituted and persisted for several days to 1 week after the drug was stopped. Two patients who had been on bed rest fainted on frequent occasions during a period of 3 to 4 days after they were allowed out of bed, even after imipramine was discontinued. Later they became symptom-free

and developed only mild symptoms on 50 mg. of the drug per day. Five patients of this group had signs and symptoms of shock and showed mental aberrations for up to 1 week from the time imipramine was stopped, after which period they recovered completely. One patient without any definite signs of shock fainted repeatedly in the bathroom. Two patients with angina pectoris and old myocardial infarctions developed new infarctions during the period of hypotension. One of these patients got out of bed against advice after imipramine had been stopped. In both patients, the occurrence of a fresh myocardial infarction should probably be attributed to the postural hypotension.

Pulse rate. Of 82 patients, 77 had no appreciable change in pulse rate in the recumbent position. One showed a slight transient increase. In 4 patients a marked increase in pulse rate was observed; 2 of these developed a myocardial infarction, a third became oliguric, and 1 had a concomitant increase with fever, which was due to the basic disease.

While standing, patients with moderate to severe postural hypotension in almost all instances developed an increase in pulse rate which disappeared after they had resumed the recumbent position and the blood pressure returned to the control level.

Electrocardiogram. Serial electrocardiograms were made on 82 patients before and during treatment with imipramine. Fifty-one electrocardiograms were normal and remained normal, with the exception of those of 2 patients, both of whom developed a left ventricular strain pattern.

Twenty-six electrocardiograms were abnormal before the study was started. Two of these showed slight to moderate improvement of T wave abnormalities, eighteen remained unchanged, and in six further abnormalities were observed. Of the six, 1 patient developed frequent premature atrial contractions and 2 manifested increased T wave inversion for no specific reason. Two electrocardiograms revealed

old myocardial infarctions. One of these showed the pattern of acute inferolateral myocardial infarction 4 weeks after imipramine was started. The other patient developed congestive heart failure with severe postural hypotension on the fifth day of medication. Although imipramine was stopped on the sixth day, the hypotension persisted. Three days later the patient got out of bed against advice and developed severe chest pain. The electrocardiogram at this time showed an extensive acute anterolateral myocardial infarction which was confirmed at the postmortem examination. The sixth patient developed congestive heart failure: the electrocardiogram showed atrial fibrillation during the third week of medication. The patient was a double amputee, and there were no changes in his daily habits or in medication prior to the development of heart failure.

There was no evidence that imipramine itself had a direct effect in altering the electrocardiogram. The changes that occurred in the cases mentioned above could be explained by such other complicating factors as hypertension, coronary arteriosclerosis, and severe myocardial abnormality.

Blood coagulation. Clotting time, bleeding time, and the one stage prothrombin time (measured in seconds) were determined prior to administration and during the course of imipramine treatment in 70, 65, and 61 patients, respectively. No significant changes were encountered, and there were no clinically notable signs or symptoms of bleeding.

Liver function. SGOT determinations (34 patients), cephalin flocculation tests (34 patients), thymol turbidity (30 patients), and alkaline phosphatase values (28 patients) failed to reveal any pathologic changes during the course of imipramine treatment.

Formed elements. Hemoglobin levels, complete white blood cell counts, and platelet counts remained unaltered in 30 patients during administration of imipramine.

Other side effects. These included papulomacular eruptions during treatment (2 patients), generalized urticaria with erythema, rise in white and eosinophil counts (in a patient who also was receiving novobiocin before the eruptions started), and agitation (3 patients). Severe hypoglycemic reactions were observed in 2 patients; however, follow-up studies in these and other patients excluded imipramine as the cause of the hypoglycemia.

Four patients of group 1 with cardiovascular diseases developed frank congestive heart failure during the administration of imipramine, 2 of whom received daily maintenance doses of digitalis. In none of these patients could a satisfactory explanation for the failure be found except that 3 of them developed severe postural hypotension before the onset of the failure. Imipramine was discontinued and a congestive heart failure regimen instituted, including strict bed rest, mercurial diuretics, and digitalis in 2 patients. Three patients recovered within 24 to 36 hours with this regimen. The fourth developed a myocardial infarction and died (see above).

In 10 patients with cardiovascular diseases, urinary sodium and potassium excretions were determined on 24 hour samples of urine during a control period and over a period of 2 to 4 weeks after imipramine administration. No significant change in the urinary electrolytes was observed in 8 patients. None of these patients developed congestive heart failure or evidence of pitting edema. One patient with nephrosclerosis and evidence of renal failure became oliguric and showed marked sodium retention which was independent of imipramine administration. The tenth patient developed urinary retention which was relieved after imipramine administration was stopped.

In 6 of these cases, decay curves of radioactive sodium (Na^{22}) were concomitantly performed before and during imipramine administration. The patient mentioned above who developed oliguria

showed marked retention of Na^{22} during the study. In the other 5 cases, imipramine had no significant influence upon the Na^{22} decay curves.

Discussion

Recently, two types of drugs have been used in the treatment of depressions: one type activates the reticular formation and thalamic projections which act together as a functional unit (the mesodiencephalic activating system[16]); the other type blocks this system.[7] The "activators" include amphetamine, pipradol, methylphenidate, iproniazid, etc. Imipramine hydrochloride belongs to the "inhibitors" of the mesodiencephalic activating system. It appears more specifically to relieve depressions but is of little value in the treatment of hyperactive psychotic patients who require tranquilization.

Side effects. Side effects have been observed frequently, but previous reports have indicated that they were seldom severe enough to interfere with treatment. A summary of untoward effects which have occurred during the treatment of 1,351 patients, as reported by various investigators,* includes the following: dryness of mouth (18.7 per cent), sweating (14.1 per cent), tremor (9.6 per cent), agitation (8.2 per cent), and nausea (5.2 per cent). Approximately 20 per cent of our patients of the younger age group complained of excessive sweating during the first 2 to 3 weeks of treatment. It was interesting to observe that most patients complained of perspiration of the face more than of other parts of the body, as was observed by other investigators.[15] Very few patients over 60 years of age experienced excessive perspiration. Dryness of mouth was present in one out of 5 patients but could only be elicited by specific questioning in most cases. Three of our 91 patients became severely agitated, requiring discontinuance of imipramine.

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*Geigy Pharmaceuticals: A comprehensive summary of data, 1959 and 1960, on Tofranil; personal communication.

Effects of imipramine on the blood pressure. Dizziness (5.2 per cent), drowsiness and fatigue (2.2 per cent), syncope (1.8 per cent), and sudden falling (1.1 per cent) have been mentioned in many reports.* However, the occurrence of hypotension (2.7 per cent) rates very low in most communications. Some observers have stated that hypotension is often noted, especially in aged and hypertensive patients.[3] These reports in most instances have failed to state whether the blood pressures were recorded in the recumbent, sitting, or standing positions. The recumbent blood pressures change little during the treatment with imipramine.

Blood pressures taken in the erect position decreased in a high percentage of patients. It should be emphasized that in 27 of the 47 patients, the decrease in blood pressures was slight, and that there were no subjective symptoms and no change in the clinical picture, so that this mild hypotension cannot be regarded as a significant side effect. Severe hypotension was observed only in patients of group 1 (10 cases) who suffered concomitantly from generalized and/or coronary atherosclerosis. Two of these 10 patients developed acute myocardial infarction, which should probably be attributed to the hypotensive episodes caused by imipramine.

Acute myocardial infarctions during the treatment with imipramine were reported by Freyhan,[5] Malitz,[13] Lehmann,[12] and Sloman.[18] The exact role of imipramine in the development of this complication is difficult to determine. Its occurrence during severe hypotensive states suggests that the drug is an important precipitating factor. It is therefore of the utmost importance to practice extreme caution in the treatment of depressive states in patients with known coronary artery disease and old myocardial infarctions.

The cause of hypotension resulting from imipramine has not been definitely determined. Hemodynamic studies recently reported on drugs with similar hypotensive action have indicated that the hypotension

is probably caused by a decrease in the cardiac output resulting from venous pooling rather than by a true vasodilator action.[1]

The occasional development of congestive failure in patients with cardiovascular diseases on imipramine is an interesting observation. Mann[14] observed 3 patients who developed congestive heart failure during the treatment with imipramine and Lehmann[12] 1 in patients with known cardiac disease. Only 2 of our 4 patients with congestive failure had episodes prior to the study, and they were receiving digitalis at the time heart failure occurred. Electrolyte studies were not available in these subjects. However, in 10 patients with compensated heart disease in whom daily electrolyte excretion studies were performed and in 6 patients on whom radioactive sodium decay curves were obtained, sodium retention could not be detected.

As in previous reports,* no significant untoward effects of imipramine on liver function, blood coagulation, or peripheral blood pattern were observed in our patients. Severe agitation in 3 patients constituted the only other important side effect necessitating discontinuance of imipramine which also has been reported previously.*

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Factors affecting the absorption and biliary excretion of erythromycin and two of its derivatives in humans

The oral administration of the propionyl ester of erythromycin and the lauryl sulfate salt of the ester produces higher serum concentrations than the parent antibiotic, erythromycin. Lee explained this by his findings in rats that the biliary excretion of erythromycin was relatively high while that of the two derivatives was low. We extended his work to humans.

In 16 patients, with bile fistula propionyl erythromycin administered orally was followed by relatively high serum and low bile antibiotic activity, as compared to erythromycin. The lauryl sulfate salt form followed a pattern similar to the ester. Diverting antibiotic-containing bile from the intestine lowered the serum antibiotic concentration during treatment with erythromycin. However, this procedure had little effect on the two derivatives, since they appear in the bile in only a minimal amount.

The clinical findings support the animal data.

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The discovery of erythromycin[4] was followed by the development of a propionyl ester of erythromycin which produces higher blood levels than the base.[1, 2, 5] Recently, an acid-stable lauryl sulfate salt of the propionyl ester of erythromycin was reported to produce serum concentrations as high when administered with food as without.[3, 4] The chemical structure of erythromycin and its two derivatives is shown in Fig. 1.[9]

Erythromycin is actively cleared from the blood by the liver. Its concentration in human liver and gall bladder bile, ranging from 2 to 50 times the blood level, indicates that an active secretory process is involved.[7, 8] In contrast, when the biliary
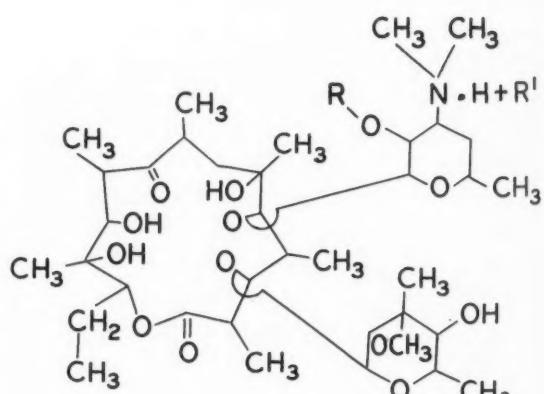
Fig. 1. Structural formula of erythromycin.

	Erythro-mycin	Propionyl erythromycin	Propionyl erythromycin lauryl sulfate
R	H	CH_3CH_2CO	CH_3CH_2CO
R[1]			$C_{12}H_{25}OSO_3$

excretion of propionyl erythromycin and propionyl erythromycin lauryl sulfate was tested in animals, relatively low bile concentrations were found. In rats, 2 hours after intraduodenal administration, the percentage of administered dose excreted in the bile was 9.2 for erythromycin, 0.57 for propionyl erythromycin, and 0.07 for the lauryl sulfate salt. The average concentration for propionyl erythromycin in bile was 64 times the serum concentration. The corresponding ratio for erythromycin was 1,400.[1]

Because of the importance of these findings in elucidating the fate of this antibiotic and its derivatives, similar studies were carried out in man. Additional investigations were included to rule out the possible influence of the presence or absence of bile salts in the intestine on the results.

Methods

Subjects with bile fistula. Sixteen patients with surgical biliary fistulas after choledochostomy were included in this study. A single 500 mg. dose of either erythromycin or propionyl erythromycin was administered during fasting to each of the 16 patients in a crossover study. Bile from the fistula was collected for antibiotic assay. Blood specimens were also obtained at intervals during bile collection. After an interval of 24 to 48 hours, the other antibiotic preparation was given and the studies repeated.

Three subjects were given propionyl erythromycin lauryl sulfate as well, and the same collections were made.

Antibiotic levels of the serum and the filtered bile were determined by a modified Rammelkamp twofold serial dilution technique with Streptococcus C203 as the test organism.[10]

To determine the effects of the bile fistula on absorption of the antibiotic, the same studies were repeated in 6 patients in the group. They received 1 Gm. iron bile salts* three times daily after meals, and the

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*Bilron.
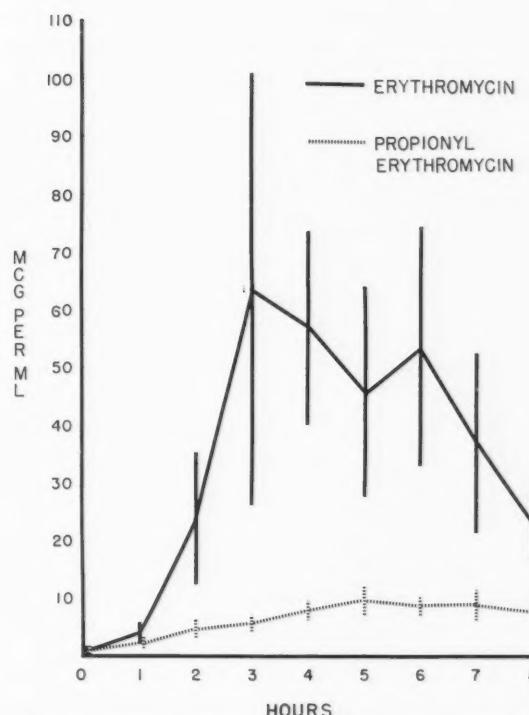
Fig. 2. Average antibiotic concentrations in bile after the administration of erythromycin and propionyl erythromycin. The vertical lines represent one standard error.
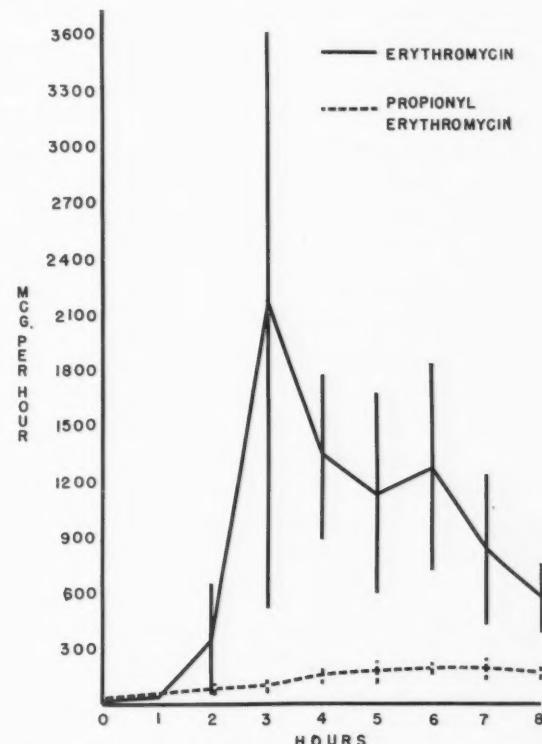
Fig. 3. Hourly antibiotic output in the bile after erythromycin and propionyl erythromycin. The vertical lines represent one standard error.

T tube was clamped to permit bile to enter the intestine for 16 hours before collection of the specimens was begun.

Normal subjects. Ten normal, fasting subjects received a single 250 mg. dose of the propionyl ester of erythromycin* orally on one day and a similar amount on another day with bile salts. The resulting serum concentrations were determined. Identical studies were carried out with erythromycin in 10 other subjects.†

Results

Subjects with bile fistula. The average bile concentration of erythromycin reached a peak, after 3 hours, of 64 μg per milliliter (Fig. 2), compared to the peak after propionyl erythromycin, at 5 hours, of 10 μg per milliliter. The differences between these results were considered significant (P < 0.01). The bile volumes during the 8 hour collection period were similar, averaging 170 ml. after the administration of erythromycin and 181 ml. after propionyl erythromycin.

The average output of antibiotic in the bile recovered by T tube drainage after erythromycin reached a peak, at 3 hours, of 2 mg. per hour. The highest value after the administration of the propionyl ester, 0.2 mg. per hour (Fig. 3), was reached at 7 hours. The differences are significant (P < 0.01). Following their administration, 1.5 per cent of the erythromycin dose and 0.22 per cent of the dose of propionyl erythromycin were secreted in the bile during the first 8 hours.

In the 3 patients who received propionyl erythromycin lauryl sulfate,‡ the antibiotic concentrations in the bile and serum were essentially the same as those after propionyl erythromycin (Fig. 5).

The average bile/serum ratio of antibiotic concentrations in 12 patients at 8 hours was 30 after the administration of erythromycin and 4 after propionyl erythromycin.

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*Ilosone.
†Ilotycin.
‡Ilosone lauryl sulfate.
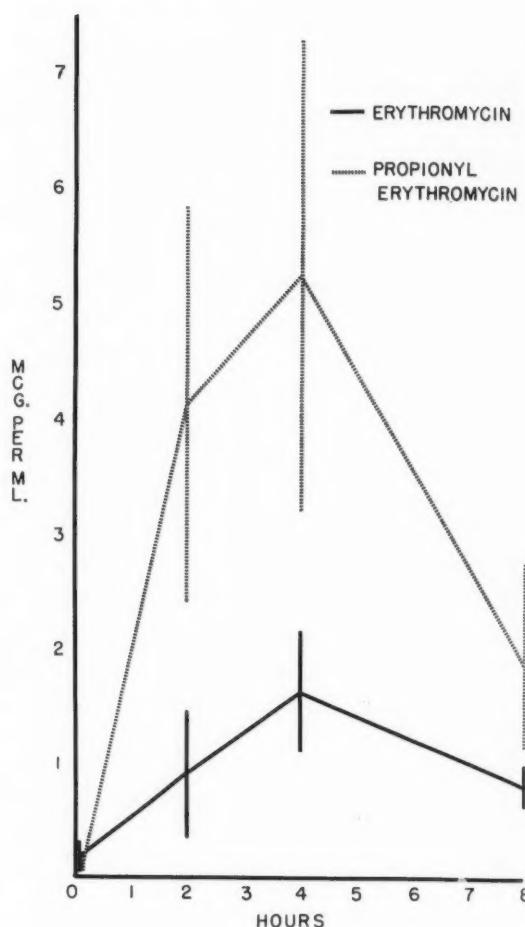
Fig. 4. Average antibiotic concentration in serum after the oral administration of erythromycin and propionyl erythromycin in 500 mg. doses. The vertical lines represent one standard error.

In 6 of the fistula patients, clamping the T tube and permitting antibiotic-containing bile to enter the intestine significantly increased the serum concentrations following the administration of erythromycin (P = 0.001), probably by permitting intestinal reabsorption (Fig. 6). The administration of iron bile salts was associated with insignificant changes in the same direction. Preventing external drainage of bile or the administration of bile salts did not influence the serum concentrations after ingestion of propionyl erythromycin.

Normal subjects. The highest average serum antibiotic concentration following the administration of erythromycin alone was 0.3 μg per milliliter. It was 0.2 μg per

milliliter after the administration of the antibiotic with bile salts. Corresponding findings in the case of propionyl erythromycin were 1 μg per milliliter and 0.7 μg per milliliter. Analysis of variance indicated that the erythromycin levels with and without bile salts were not significantly different.

Discussion

The previously cited animal data indicating a quantitative difference between the biliary secretion of erythromycin and the propionyl ester of erythromycin has been confirmed in humans. The results with the lauryl sulfate salt of propionyl erythromycin do not differ from those with propionyl erythromycin itself. In patients, erythromycin was secreted 10 times as rapidly as propionyl erythromycin; in rats, 20 times.

The preferential secretion of erythromycin in the bile appeared to be one explanation for the lower serum levels of erythromycin observed. The fate of antibiotics re-entering the intestine via the bile is presumed to be similar to that of those ingested orally—only a small proportion is absorbed. While it follows that the greater

the proportion of the ingested dose of an antibiotic which is excreted in the bile, the less will remain in the blood, the proportion of the ingested dose which was secreted in the bile was small with each of the three antibiotics, and the total amounts involved were not sufficient to account entirely for the differences in serum concentrations obtained.
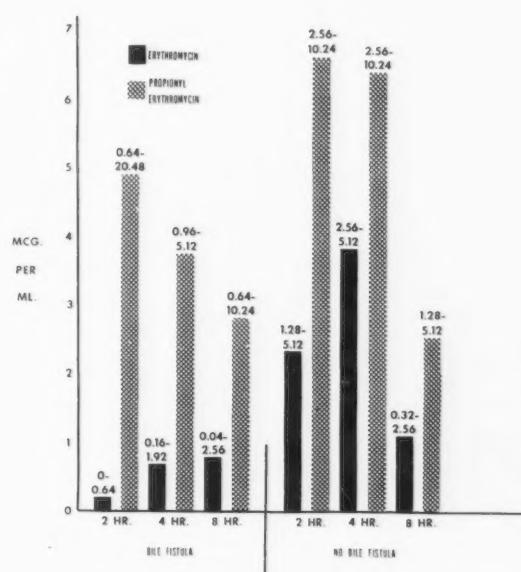
Fig. 6. Effect of diversion of bile from the intestines on absorption of erythromycin and propionyl erythromycin, and average antibiotic concentrations and range in serum in 6 subjects.

Conclusions

In 16 patients with bile fistula, the biliary excretion of erythromycin was high, while that of propionyl erythromycin and propionyl erythromycin lauryl sulfate was low. The difference in biliary secretion appeared to explain, at least in part, the higher serum concentrations that resulted when propionyl erythromycin or propionyl erythromycin lauryl sulfate was administered.

Fig. 5. Maximum serum and bile concentrations of antibiotic after the oral administration of erythromycin and two derivatives in 3 subjects with fistula. The vertical lines represent one standard error.

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Clinical pharmacology of hypnotics and sedatives

If there is any tendency to become complacent about the adequacy of knowledge in a given area, a review of the subject usually serves to dispel this attitude. Although there exists a formidable body of knowledge on the pharmacology of the hypnotics and sedatives, that which remains unknown is even more formidable. Many of the experiments which have yielded information on the pharmacodynamics and disposition of these drugs in laboratory animals do not have their counterparts in human studies. In such instances, only a logical guess can be made in an effort to bridge this gap. The known effects of the barbiturates, bromides, paraldehyde, chloral hydrate, and newer hypnotics and sedatives in man are presented and an attempt is made to compare these with findings in other species. Where data of a critical nature with respect to the human pharmacology of these drugs are lacking, the fundamental features of their actions on other animals are presented.

In addition to the more classic aspects of their pharmacodynamics, the more significant alterations in behavior produced by these agents are summarized. Studies dealing with their pharmacology in both the normal and pathologic states and in therapeutic and toxic dosages are outlined. Finally, the disposition of these compounds in the body is considered, inasmuch as this aspect of their pharmacology may have important bearing on the responses elicited by them in the organism.

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The Council on Drugs of the American Medical Association has designated hypnotics and sedatives as nonselective depressants of the central nervous system. It has also pointed out that a number of drugs, e.g., tranquilizers and muscle relaxants, which are pharmacologically dissimilar to this group of agents, may result in responses broadly interpreted as sedation. Inasmuch as this review has a pharmacologic rather than a psychologic orientation, only those drugs which display hypnotic as well as sedative activity and which, from the classic standpoint, are so classified will be considered. Major attention will be given to their effects in man, and, insofar as possible, these will be compared and contrasted with observations of a similar nature in other species. Whereas it has not been the author's purpose to provide a compendium of the entire clinical literature, it is hoped that this review will serve as a source of most of the presently documented and well-controlled studies of the

pharmacology of the hypnotics and sedatives in man.

The drugs to be considered in this review, their doses, onsets and durations of action, and indications for use have been adequately discussed previously.[72] Detailing of their chemical and physical properties may be found in any good standard textbook of pharmacology or other appropriate reference source, e.g., *The Merck Index*.

Central nervous system

Behavioral effects. As a result of the recent promotion of and interest in the "tranquilizing" drugs, there has been a spate of papers dealing with the behavioral effects of the hypnotics and sedatives. The majority of such studies have concerned themselves with the barbiturates, and many of these have involved comparisons of the latter with tranquilizers, e.g., reserpine, chlorpromazine, etc. Critical evaluations of a variety of behavioral effects have been made in both the absence and presence of disease, and they will be discussed in that order. Inasmuch as most of the earlier studies relating to such effects were inadequately conceived and designed or poorly controlled, attention will be focused on the more recent endeavors in this area.

The many diverse behavioral effects of the barbiturates which have been observed are in agreement with the generally accepted belief that this class of drugs acts at all segmental levels and at all levels of functional organization of the central nervous system, with the cerebral cortex perhaps being most sensitive to their depressant effects.[80] Von Felsinger, Lasagna, and Beecher[190] determined the effects of 0.1 Gm. of orally administered pentobarbital sodium on a number of complex psychologic functions in a group of healthy male college students. Visual perception, attention, arithmetic performance, and recall were significantly impaired as long as 5½ to 8 hours after ingestion of the drug. Associations also were found to be increased in number, but these were less

"reality determined," i.e., showed less relation to external stimuli, than after placebo. The drug tended to facilitate resistance to distraction but had no effect on serial learning and analogy tests. They offered these findings as objective evidence of the frequently described "hangover" phenomenon. Other workers[113] have demonstrated the effects of a hypnotic dose of one of the barbiturates on arithmetic performance. Seventy-five minutes after oral administration of 200 mg. of secobarbital, ability to add digits was impaired, as were capacities to do digit-symbol and pursuit rotor tests. These authors have concluded that such effects are due not only to the specific pharmacologic activity of the drug but also to the specific reactivity of the subject and the interaction of the two. No correlation was observed between subjective and objective psychologic effects of a given drug (other agents were included in this study), but drugs which produced the greater mean objective effect also resulted in the greater mean subjective effect. In a comparison of the effects of 100 and 200 mg. of secobarbital, Kornetsky and Humphries[114] found that 90 minutes after the ingestion of drug, the larger dose significantly impaired performance on digit-symbol, tapping speed, and pursuit rotor tests, whereas the smaller dose did not. Other workers[13] have shown that 300 mg. amobarbital administered orally in three divided doses within 4 hours prior to testing particularly impairs the most elementary of sensory-motor functions and negligibly alters intellectual functions. The findings of Goldstein, Searle, and Schimke[78] are essentially in agreement with those of Kornetsky, Humphries, and Evarts[113] with respect to the effects of 200 mg. of secobarbital on psychomotor performance. The former found that in a group of medical students, the barbiturate significantly impaired performance in all (simple and contingent reaction times, computation, repetitive coordination, hand steadiness, repetitive movement) but one (tachistoscopic digit recognition) of the tests employed. These disruptive effects on

different types of learned behavior are not dissimilar to those described by Loomis and West,[127] who observed that 100 mg. of secobarbital produced a significant impairment of performance on a simulated automobile driving test apparatus. In a similar type of test (pursuit rotor), it has been observed that a larger dose (250 mg.) of amobarbital sodium also decreases performance.[57] Goldstein, Searle, and Schimke[78] have emphasized the differential effects of secobarbital on complex and simple reaction times, the former affected more than the latter. They also have pointed out that the only test they employed which was unaffected by the barbiturate differed from the others in that the subject had only to recognize familiar symbols but not to make any unusual motor responses or perform stimulus-cued chores of alternative responses, neither was a high rate of response demanded. Similar differential actions in man have been reported by Goodnow and associates,[81] Von Felsinger, Lasagna, and Beecher,[191] and Kornetsky and Humphries.[114] Comparable results have been obtained by Dews[45] in studies on three types of operant behavior in pigeons.

In line with other studies[57, 127] dealing with the effect of barbiturates on a complex perceptual motor task, Mirsky, Primac, and Bates[137] have been able to show that 200 mg. of secobarbital has a deleterious effect on continuous performance in a test designed to measure deficit in sustained attention. Significant impairment occurred only between 30 and 75 minutes after drug administration, no significant alterations being observed from 75 minutes to 175 minutes following the drug. Qualitatively similar effects were obtained after chlorpromazine. They interpret these results to mean a disturbance by the drugs of the subcortical activating system. Similar conclusions have been reached by Callaway,[32] who found a broadening of attention as measured by the Stroop color-word interference test or by Witkin's colored embedded figure test 1 hour after ingestion of 265 mg. of amobarbital.

The duration of action of barbiturates, in terms of behavioral effects, would appear to be influenced by a number of factors. Among these is the nature of the behavior being evaluated. Whereas Mirsky, Primac, and Bates[137] found 200 mg. of secobarbital significantly affected sustained attention only between 30 and 75 minutes after the drug, Von Felsinger, Lasagna, and Beecher[190] demonstrated an impairment in visual perception, attention, arithmetic performance, and recall as long as 8 hours after administration of the barbiturate. The reasons for the differences in duration of effect of the two drugs on attention cannot be stated with assurance. They may be related to differences in testing procedures, inherent differences in the durations of action, or some other factor(s). Goodnow and colleagues[81] found one measure of psychologic performance, i.e., tapping speed, significantly impaired as long as 14 hours after oral administration of 100 mg. of pentobarbital sodium. The data of Kornetsky, Vates, and Kessler[115] are essentially in agreement with this observation. From 14 to 15 hours after the ingestion of 200 mg. of secobarbital, the performance of 18 subjects on a series of three tests (digit-symbol substitution, symbol copying, tapping speed) was still impaired. Similar findings were obtained with chlorpromazine (100 or 200 mg.) but not with a smaller dose (100 mg.) of secobarbital. These data provide further objective evidence in support of the clinical impression of the hangover effect the morning after barbiturate medication and demonstrate a similar potentiality for chlorpromazine.

On the basis of maze and conditioned reflex measures, the sedatives and hypnotics (barbiturates, bromides, chloral hydrate) appear to have a consistently detrimental effect on learning by laboratory animals.[19] In man, the rate of learning a simple task is not slowed significantly 2 hours after oral administration of 60 or 120 mg. of phenobarbital.[112] In these same studies, motor coordination and reaction

time, as measured by a simple motor response and a choice visual motor response, respectively, were similarly unaffected. However, sufficiently large doses of phenobarbital or any other hypnotic could be expected to produce effects on learning in man comparable to those observed in other animals.

The effects of various hypnotics on conditioned responses in laboratory animals have been the subject of a number of studies. As an example, the work of Cook and Weidley[38] might be cited. They demonstrated a nonspecific block of a conditioned response in the rat by barbital and methylparafynol. Pentobarbital was capable of producing a moderate degree of specific block of the conditioned response, but only after administration of doses sufficient to produce gross changes in behavior, e.g., ataxia. Intravenous administration of amobarbital (180 to 360 mg.) in man reduces the number of conditioned responses (eyelid conditioning) during both acquisition and extinction.[67] In studies[164] on the conditioned galvanic skin response in normal individuals, the intramuscular administration of 200 mg. of amobarbital sodium 2 hours prior to testing had no effect on the unconditioned response (the shock) but did inhibit spontaneous recovery of the galvanic skin reflex. Unfortunately, there has been little effort to approach these aspects of the behavioral effects of hypnotics (as well as other depressants of the central nervous system) in an organized manner and to establish correlations between responses in man and other animals.

An interesting study of subjective effects of hypnotics has been reported by Smith and Beecher.[175, 176] In a group of college athletes, 100 mg. of secobarbital produced distortion in judgment. A group of swimmers believed their performance to be unusually good whereas in fact it had been significantly impaired. This dose also produced intoxication, elation, and deactivation, whereas 50 mg. of the drug resulted only in elation and activation. Barbiturates in appropriate dosage also appear to affect

judgment with respect to time.[79] After ingestion of 200 mg. of secobarbital, normal individuals experienced an alteration in the apparent duration of an auditory stimulus in the direction of underestimation. This change became significant 1 hour after the drug and was compatible with the feeling that time seemed to be "flying."

Certain perceptual studies[58, 59] with respect to vision have demonstrated alterations following barbiturate administration. Within 1 hour after ingestion of amobarbital (270 mg.), the threshold for perception of a visual stimulus (a red light) is lowered if this stimulus is followed by a flash of white light of short duration but not if the duration of the white stimulus is long. The duration of visual aftereffects also is reduced. This drug also has been shown to lower critical frequency of flicker[3] in doses of approximately the same magnitude. Perception of cutaneous tactile stimuli is adversely affected by barbiturate administration.[64] Injection of amobarbital intravenously in amounts sufficient to produce slurred speech, nystagmus, ataxia, and drowsiness causes errors in perception of simultaneous stimuli, i.e., only one of two stimuli is perceived.

It has been a tacit assumption that the depressant effects of alcohol summate with those of the barbiturates. A recent study[62] has demonstrated the additive nature of this relationship in mice. That the situation is more complex when one attempts to evaluate the behavioral effects of such a combination in man is suggested by the experiments of Joyce and associates.[103] Although their findings lack statistical significance, they are interesting and suggestive. Complex reaction times of 8 young male subjects were determined at two different times after the ingestion of 60 ml. of alcohol, 130 mg. of phenobarbital or 30 ml. of alcohol and 65 mg. of phenobarbital, or placebo. The tests were conducted 35 minutes after the alcohol and 75 minutes after the barbiturate and again 2 hours after the first tests. Mean reaction time decreased whether or not the subjects received drugs.

Three hours after treatment, the decrease was smaller after phenobarbital and greater after alcohol or both drugs than after placebo. In typing tests, subjects under the influence of phenobarbital typed more slowly, made fewer errors, and corrected more errors than following placebo. Alcohol administration was followed by faster typing with more errors. When the drugs were combined, the tendencies shown after only alcohol were increased. The authors were of the opinion that the barbiturate potentiated some of the effects of alcohol.

Of the many publications describing behavioral studies in individuals with neuroses, psychoses, or psychosomatic disorders, few are sufficiently well designed or critically objective to warrant consideration. Methylparafynol in a dose of 2 Gm. per day has been reported[8] to suppress eyelid conditioning and sympathetic conditioning as measured by the psychogalvanic response. Chronic administration of a bromide preparation* in sufficient dosage to produce blood bromide levels of 0.40 to 0.76 mg. per 100 ml. in anxious patients significantly impaired reaction at high speed in a driving test.[189] There was also a significant reduction in visual acuity and a tendency, although not significant, toward decreased accuracy. In normal individuals, Grunewald[87] observed that hexobarbital, when given intravenously in one-tenth the dose required to produce anesthesia, caused a slight reduction in reaction time to auditory or visual stimuli. In mentally ill patients with normal reaction times, the barbiturate was without effect. In similar patients having significantly prolonged control reaction times, the response to hexobarbital appeared to be dependent on the type of mental disturbance. No logical explanation for these differences in effects was apparent. Impairment of the mental speed of schizophrenic persons has been reported as a consequence of the ingestion of 360 mg. of amobarbital.[23] It is interesting to note that in a well-designed comparative study by Benn and Ellis[12] on the control of disturbed behavior in a group of psychotic patients, chlorpromazine (150 mg. per day), phenobarbital (360 mg. per day), and placebo did not differ significantly.

The "sedation threshold test" has been proposed and employed[106, 171] as a means of assessing degree of tension and for various types of experimental studies in psychotic patients. However, Ackner and Pampiglione[1] have made a very careful evaluation of this test and have been unable to demonstrate that the results obtained from its use are correlated with anxiety ratings or diagnostic groupings. Thus it appears that any studies which employ this technique are of doubtful significance.

Sedative and hypnotic effects. With the exception of the bromides, all members of this group are rather widely used for the production of hypnosis as well as sedation. The necessity of prolonged usage to obtain sufficient concentrations of bromide ion in the body to effect sleep precludes the satisfactory use of bromide-containing preparations for this purpose. Otherwise, all these agents resemble one another qualitatively but exhibit marked quantitative differences. These relate particularly to potency and onset and duration of action. Although many comparative clinical studies have been conducted in an attempt to establish the quantitative nature of these differences, few can claim the essential scientific objectivity and design to make the data meaningful. This applies in particular to such comparisons as dosage and dosage schedule in measurements of sedative activity. For this reason, it would serve no useful purpose to cite work in which this has been the stated objective. Although it would appear that comparisons of hypnotic activity might have greater significance, since a more precise and objective definition of end point should be achievable, such does not appear to be the case. In one carefully controlled study,[120]

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*Tranquil.

the hypnotic effects of three agents, chloral hydrate, pentobarbital sodium, and methylparafynol, were compared in 268 patients. Chloral hydrate in a dose of 1.0 Gm. produced rapid induction of sleep but was no different than a placebo in producing uninterrupted sleep. In a dose of 100 mg., pentobarbital appeared to be somewhat less effective than 1 Gm. of chloral hydrate in inducing sleep, but doubling the dose resulted in the satisfactory induction and maintenance of sleep. Doses of methylparafynol as great as 1 Gm. were indistinguishable from a placebo. This study indicates pentobarbital to be somewhat less than 10 times as potent as chloral hydrate, which, in turn, is more potent than methylparafynol. Thomson[183] has compared the hypnotic activity of the latter drug with secobarbital in hospital patients by a method of sequential analysis and has found it to be about one-fourth as effective as the barbiturate, i.e., 100 mg. of the latter was indistinguishable from 400 mg. of the former. The hypnotic doses of pentobarbital and secobarbital are stated to be the same (0.1 Gm.),[72] and experience indicates the drugs are equally effective. One then wonders which of these comparisons is valid. It can only be said that conflicting reports of this type indicate the need for a more careful examination of the criteria which are employed to assess the clinical effectiveness of hypnotic agents and the establishment of more rigid and standardized procedures for the realization of quantitatively meaningful data.

Analgesia. This group of drugs differs markedly from the analgesics in that they fail to significantly affect pain perception in the absence of significant alterations in other modalities of sensation. Wolff, Hardy, and Goodell[203] have shown that a barbiturate in a dose not quite sufficient to cause loss of consciousness produces only a slight (20 per cent) elevation of the cutaneous pain threshold. Although the barbiturates are not analgesics in the true sense, since they are relatively ineffective for the relief of moderate to severe pain when given alone, Keats and Beecher[107] were able to demonstrate relief of postoperative pain in 50 per cent of those patients who received a hypnotic dose (60 or 90 mg. per 70 kg. of body weight) of pentobarbital sodium by the intravenous route. This was significantly better than the results achieved with saline (20 per cent relief) but not as good as that realized with morphine (80 per cent relief). The authors suggested the results with the barbiturate might be explained by depression of the internuncial spread of pain impulses and suppression of the psychic phase of pain experience. With respect to the latter, it was further suggested that there may be a decreased concern about pain rather than a reduction in pain perception, a situation analogous to that occurring after frontal lobotomy. Hill, Belleville, and Wikler[94] have attempted to examine the psychic element as a factor in the relief of experimental pain. Subjects (former drug addicts) were submitted to a test situation in which they received a shock if their responses were not sufficiently rapid. Repeated shocking, as a result of inadequate performance, produced a striking disruption of performance which became apparent as a marked slowing of the reaction time. Administration of 200 mg. of pentobarbital intramuscularly had no effect on the altered performance, whereas morphine (15 mg.) administered by the same route markedly reduced the disrupted performance and even prevented the effect of shock on reaction time. It was concluded that morphine, in some way, relieved the anxiety associated with the anticipation of pain, an action not shared by the barbiturate. This property of morphine, they state, is a necessary characteristic of a potent analgesic, but the possibility that barbiturates may act similarly in situations where pain is less severe and anxiety is of lesser degree is not excluded.

Anticonvulsant actions. The capacity of anesthetic doses of barbiturates and certain other hypnotics to suppress drug-induced convulsions and the convulsions of tetanus and status epilepticus is well recognized.

Of the commonly employed agents in this group of drugs, however, only phenobarbital, metharbital, and mephobarbital show, with bromides, the selective anticonvulsant activity which has made the latter useful in the symptomatic treatment of epilepsy. The anticonvulsant activity, particularly with respect to electroencephalographic effects, of barbiturates has been studied rather extensively. The latter studies, prior to 1949, have been adequately summarized in the review by Toman and Davis.[187] Inasmuch as the drugs which are effective in the treatment of epilepsy need not have a depressant effect on the central nervous system, it comes as no surprise that with the use of these as antiepileptic agents, there are associated no common electroencephalographic changes. For a discussion of the theoretic considerations relating to the mechanism of action of these agents as antiepileptics, the reader is referred to the publications by Toman and Goodman[186] and Toman and Taylor.[188] The specific capacity of phenobarbital to depress the motor cortex has been extensively studied. Early work by Keller and Fulton[108] showed phenobarbital to be the only one of several barbiturates studied capable of completely suppressing excitability of the motor cortex of monkeys in anesthetic doses. This activity was not shared by chloral hydrate or tribromoethanol. It raises the threshold for electroshock seizures in normal animals as well as animals rendered more susceptible to such convulsions by hyponatremia. It also abolishes the tonic extensor phase of maximal electroseizure but has only a slight protective effect against pentylenetetrazol*-induced convulsions. The therapeutic index in most of these tests is relatively low since doses only slightly greater than those producing significant effects on the seizures result in more generalized depression of the central nervous system. The mechanism by which phenobarbital reduces the excitability of the motor cortex and pre-

vents the spread of seizure discharges is unknown. Unlike the oxazolidine-2,4-diones, it lacks the capacity to abolish, in a selective manner, the three per second spikedome dysrhythmias characteristic of petit mal.

Despite the many studies on barbiturates structurally similar to phenobarbital, only two, metharbital and mephobarbital, approach it in antiepileptic activity. Mephobarbital closely resembles phenobarbital in its effects on electrically or chemically induced convulsions in the laboratory but is somewhat less potent, whereas metharbital is less effective against electroshock convulsions and more effective against pentylenetetrazol convulsions than is phenobarbital.[54] Both drugs are reported to produce less sedation when employed in therapeutically effective doses than does phenobarbital.

Administration of bromides does not result in protection against experimentally produced convulsions until other manifestations of depression of the central nervous system become evident.[184] Similarly, maximal electroshock seizures in psychiatric patients who are subjected to electroshock therapy are modified only by doses beyond the usual therapeutic range.[185]

Since epileptic seizures can be prevented by drugs in the absence of any significant alteration in the electroencephalogram,[46] it would make no sense to indulge in an attempt to correlate the electroencephalographic effects of these drugs with their therapeutic effectiveness in epilepsy. Such alterations in the electrical activity of the central nervous system which they have been observed to produce will be considered in the following section.

Electroencephalographic effects. The literature dealing with this aspect of the actions of the hypnotics and sedatives is sufficiently extensive to preclude detailed consideration in this review. Surface as well as depth recordings of electrical activity in the central nervous system have been obtained after a number of drugs and under a variety of conditions. The greatest

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*Metrazol.

amount of information of this nature has been derived from studies with the barbiturates, and much of this has been summarized by Toman and Davis.[187] Electroencephalographic records obtained after hypnotic doses of the various barbiturates are consonant with those of natural sleep. Most studies have emphasized the occurrence of rapid activity preceding the appearance of normal sleep patterns. The rapid activity, ranging between 20 and 30 cycles per second and most pronounced in frontal and parietal leads after intravenous pentobarbital or secobarbital, is not demonstrable in all individuals.[121] The observed effects are influenced by, among other things, the dosage and the time interval before recording. Using thiopental, Brazier[21] routinely observed mental clouding coincident with the appearance of rapid activity. Slow activity appeared only with loss of consciousness, at which time the rapid activity disappeared. Persistence of activity in the alpha frequency persisted during unconsciousness. During sleep induced by pentobarbital (90 mg.), the incidence of such activity has been related to the incidence of movements by the subject.[22] Occurrence of rapid activity has been observed following intracarotid injection of amobarbital (75 mg.), appearing not less than 20 seconds after the drug.[197] Behavioral changes (hemimotor, hemisensory, and homonymous hemianopic defects) were predominantly unilateral and on the opposite side, whereas the electroencephalographic changes were, for the most part, bilateral. Certain behavioral changes (nystagmus, dysarthria, sleepiness), as well as electroencephalographic rapid activity and sleep patterns which follow intravenous amobarbital, were never seen as initial responses to intracarotid amobarbital. Toman and Davis[187] have adequately summarized the results of studies in man on the electroencephalographic changes induced by barbiturates. They state that there is "a general but not invariable appearance of fast activity or increase of beta activity after small doses

which produce minimal psychic effects, whereas larger doses sufficient to produce sleep alter the record in the direction of dominant slow rhythms with the appearance of spindles and other features of the EEG in natural sleep."

Information obtained from examination of changes in electrical activity deeper in the central nervous system (depth electrogram) has not been too fruitful in providing much more than a more precise description of such altered manifestations of function. As an example, the work of Monroe and associates[138] might be cited. They obtained recordings from electrodes implanted in the septal region, hippocampus, amygdaloid nucleus, caudate nucleus, hypothalamus, and tegmentum of the mesencephalon. Administration of amobarbital resulted in homologous changes in cortical and subcortical activities similar to those recorded from the usual scalp electrodes. The subcortical changes characteristic of sleep were most obvious in the septal and hippocampal regions, and rarely were such patterns seen in subcortical structures before they were apparent in the cortex. Again Toman and Davis[187] may be quoted with reference to the physiologic mechanisms involved. They state that the descriptive studies on electrical activity are not "incompatible with the view that the essential mechanisms of barbiturate action are increased recovery time and increased threshold for cerebral neurons in general, with a somewhat greater sensitivity of cortex than of diencephalic centers." It is their belief that the variety of qualitative changes produced in the electroencephalogram by barbiturates is an expression of the functional organization of the brain rather than a manifestation of an additional type of drug action.

One can establish a general correlation between electroencephalographic changes, psychic alterations, and serum bromide level, but considerable variation exists between individuals.[83] Eighteen per cent of a series of patients exhibited some EEG abnormalities with a serum bromide con-

centration of 100 mg. per 100 ml. At a level of 200 mg. per 100 ml. or greater, the incidence was 88 per cent. At levels above 200 mg. per 100 ml., there usually was observed diffuse, slow activity typically associated with irregular, high voltage, 2 to 5 per second waves. Psychic changes were marked at these levels. Rapid activity was mixed with the slow component when the serum bromide level was 180 mg. per 100 ml., and at 160 mg. per 100 ml., the frequency was similar to that seen in moderate degrees of barbiturate poisoning, mainly in the 12 to 25 per second range. Rapid activity appeared as the serum level of bromide fell and as the mental status of the patients improved.

Chloral hydrate and paraldehyde qualitatively resemble the barbiturates in their effects on the EEG.[187] Less pronounced effects occur after hypnotic doses of chloral hydrate than following barbiturates,[83] and paraldehyde is more effective in raising the threshold for electrically induced convulsions in animals than is chloral hydrate. The latter observation is compatible with the greater clinical effectiveness of paraldehyde as an anticonvulsant. Administration of methyprylon to dogs produces EEG changes similar to those occurring after pentobarbital,[46] and it would be expected that this drug and the newer hypnotics would be very much alike in terms of EEG effects in man.

Spinal cord. Only mention will be made of actions of hypnotics at this level since it is undoubtedly safe to say that virtually all definitive information of this nature has derived from studies on species other than man. It is well known that barbiturates can increase several fold the threshold of response to sensory stimuli. Adequate doses will markedly depress all spinal reflexes.[199] With smaller doses, however, selective depression of internuncial neurons may result. Brooks and Eccles[27] have obtained evidence from studies on the monosynaptic pathway that the local increase in threshold of the motor neuron produced by barbiturates is the result of an increased elec-

trical resistance of the cell membrane. On the basis of available evidence from laboratory experiments, one is led to conclude that the effects of barbiturates (and perhaps the other hypnotics) on the spinal cord (also peripheral nerves) are not too unlike those higher in the neuraxis, but the sensitivities of the latter are much greater.

Other effects. Nystagmus after intravenous administration of barbiturates may occur.[46] It was observed in 50 per cent of a group of normal individuals following the intravenous injection of an appropriate dose of hexobarbital.[15] The presence of head injuries apparently increases the facility with which it can be produced, and the duration is prolonged in individuals with objective evidence of cerebral lesions. Normally, the eye follows a moving object with a smooth pursuing movement; studies[153] with thiopental (100 mg.) administered slowly by the intravenous route have shown this smooth movement to be converted to a succession of jerks, each one refixating the escaping object. In the presence of nystagmus, barbiturates may have an opposite effect.[142] The slow intravenous administration of 50 to 150 mg. of amobarbital has been shown to abolish the nystagmus and the associated blurring of vision and/or oscillopsia occurring in patients with multiple sclerosis. Pupil size and speed of pupillary response under light and dark stimulus conditions have been found to be unaltered 2 to 3 hours following the oral administration of secobarbital (100 and 200 mg.).[34]

Transient astereognosis may be produced by intravenously administered amobarbital.[150] Patients with unilateral brain lesions and who exhibit some degree of defect in somatic sensation but no impairment of stereognosis became astereognostic after sufficient barbiturate was administered to produce nystagmus and dysarthria. Patients with tetanus exhibit repetitive spontaneous electromyographic activity between spasms, and this activity is reduced by pentobarbital (200 mg. intramuscularly).[149]

The antispasmodic activity and elevation of the threshold by the barbiturate for reflex spasm induced by various types of stimuli appear to be roughly proportional to the soporific effect produced. Abnormal involuntary movements, e.g., resting tremors, intention tremors, athetoid, choreic, and dystonic movements, are affected in varying degrees by intravenous administration of amobarbital and can be grouped on the basis of the amount of barbiturate required to abolish the movement.[13] Apparently latent motor weaknesses in patients with lesions involving parts of the central nervous system which mediate motor functions can be brought out by doses of a barbiturate which do not similarly affect normal individuals.[182] Antipyretic activity has been demonstrated for a derivative of barbital, i.e., 1-phenyl-5,5-diethyl-barbituric acid,* and it appears to be superior to phenobarbital in controlling febrile convulsions in children.[136]

Respiration

Precise studies on the respiratory effects of the hypnotics and sedatives in man are not plentiful. Most of our information on their comparative activities and the details of their actions derives from laboratory data. The barbiturates are progressive depressants of respiration, the degree depending on the dose. Orally administered hypnotic doses produce a mild decrease in respiratory activity not unlike that occurring with sleep. Notable respiratory depression was observed in only 3 per cent of a series of 97 patients who received 50 to 150 mg. of secobarbital intramuscularly prior to induction of anesthesia.[50] Oral administration of methyprylon in the upper range of hypnotic dosage (400 mg.) has been shown to cause a slight but significant increase in the carbon dioxide tension of arterial plasma.[132] This can be interpreted as a reflection of a mild degree of respiratory depression. That hypnotic doses (100 or 200 mg.) of secobarbital do not alter

the response of the respiratory minute volume to carbon dioxide is suggested by the work of Eckenhoff, Helrich, and Hege.[49] Inasmuch as only 3 subjects were employed in this study, it requires confirmation. In patients with impaired respiratory function (severe pulmonary emphysema), hypnotic doses of barbiturates may have more marked effects on respiration. Administration of 100 mg. of secobarbital to such individuals resulted in a reduction in respiratory minute volume, an increase in carbon dioxide tension, and a decrease in the pH and oxygen saturation of arterial blood.[201] A large hypnotic dose (200 mg.) of pentobarbital will reduce respiratory minute volume about 10 per cent, but comparable depressant doses of paraldehyde are stated not to affect respiration significantly.[80] Amounts of barbiturates sufficient to produce anesthesia cause a greater degree of respiratory depression. Respiratory minute volume is significantly depressed during thiopental anesthesia,[146] the degree of depression dependent on the depth of anesthesia. Oxygen concentrations of arterial blood were maintained at high levels under the conditions of these studies, and respiration could still be stimulated by administration of 5 per cent carbon dioxide, the response being greater at lighter than at deeper levels of anesthesia. Depression of the respiration is the major danger in acute intoxication with the barbiturates, and death usually is due to respiratory failure. Reduction of respiratory activity usually is manifested by a decrease in depth which is mainly responsible for the reduction in respiratory exchange. Changes in rate are not uniform and are determined to a great extent by the carbon dioxide tension in the blood. Susceptibility of the respiratory center to stimulation, even after spontaneous respiration has ceased, makes the use of respiratory stimulants logical in acute poisoning by the barbiturates, even when pulmonary ventilation is markedly decreased.[89] Although respiratory depression is one of the major features of acute intoxication with the hypnotics,

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*Pyrictal.

occasionally an exception is found. Certain reports[74, 105] indicate severe poisoning with glutethimide can be present without marked impairment of respiration.

Since peripheral chemoreceptor mechanisms are less sensitive to the depressant effects of hypnotics (barbiturates) than is the respiratory center, centrally directed impulses, deriving from stimuli in sino-aortic areas, may maintain respiration in the presence of a markedly depressed center. Under such circumstances, the center may be not only insensitive to carbon dioxide but further depressed by inspiration of high concentrations of this gas at a time when respiration is being maintained mainly by the peripheral stimulant effect of anoxia.[9] Removal of the anoxic stimulus by administration of oxygen can result in further depression or cessation of respiration. Thus, while the respiratory center still responds to carbon dioxide, i.e., during the lesser degrees of depression of the central nervous system, respiration is regulated mainly by the tension of this gas in the blood but in severe degrees of depression respiratory activity is effected primarily by the anoxic drive mediated by peripheral chemoreceptor mechanisms.

Respiration also may be embarrassed as a consequence of hiccoughing, coughing, and laryngospasm, particularly when the short-acting and ultra short–acting barbiturates are administered intravenously in the absence of appropriate premedication. On the basis of studies in cats by Burstein and Rovenstine,[28] it has been assumed that this is the result of heightened central parasympathetic activity produced by the barbiturates. More recently, direct recordings from the muscles of the glottis in the goat have failed to demonstrate such an action for either pentobarbital or thiopental.[141] In acute poisoning with the barbiturates, pulmonary edema and hypostatic pneumonia may further embarrass respiration and complicate the respiratory picture. That a similar situation can arise with the newer agents is exemplified by a fatal case of poisoning with methyprylon.[155]

Cardiovascular system

In the therapeutic doses employed for their sedative or somnifacient actions, these drugs do not greatly affect the cardiovascular system. As a result of the slight reduction in irritability and reactivity of the central nervous system, blood pressure and heart rate may be decreased somewhat, quantitatively similar to that occurring at rest or during natural sleep. However, even small doses of the barbiturates, when injected rapidly by the intravenous route, can produce abrupt but transient hypotensive responses and large doses, 70 to 90 mg. of thiopental or pentobarbital per kilogram (greater than those required to produce deep anesthesia), in artificially respired dogs cause severe hypotension.[42] In the presence of hypertension, significant reduction of systolic blood pressure without a concomitant decrease in diastolic pressure has been reported[41] after administration of small doses of a barbiturate. A decrease in diastolic as well as systolic blood pressure following intravenous administration of amobarbital to hypertensive patients also has been observed.[116] Associated with the decrease in blood pressure there usually was a reduction in total peripheral resistance. On the other hand, anesthetic doses of barbiturates in normotensive individuals have been found[102, 124] to increase or have no effect on total peripheral resistance. Cerebral blood flow is well maintained during thiopental anesthesia even though the oxygen consumption and the respiratory quotient of the brain are significantly depressed.[196] When smaller amounts (insufficient to produce anesthesia) of this drug[109] or phenobarbital[131] are given, no measurable changes in cerebral blood flow or cerebral metabolism occur. Other observations,[131] made under thiopental or amobarbital anesthesia in individuals with toxemia of pregnancy, indicate that in addition to a decrease in oxygen utilization by the brain there is a significant reduction of cerebral blood flow. The blood supply to the kidney, like that to the brain, appears to be little affected

by sedative doses of a barbiturate (phenobarbital) or chloral hydrate.[95] Similarly, anesthesia with pentobarbital produces no significant changes in the splanchnic blood flow in dogs.[55]

The capacity of the barbiturates to reduce cardiac output, when given in sufficient amounts to experimental animals, is well known. In the heart-lung preparation of the dog, 200 to 225 mg. of thiopental or pentobarbital reduces output as much as 75 per cent.[42] Utilizing the dye dilution technique for measurement of cardiac output in man, Etsten and Li[53] have found, however, that the intravenous administration of thiopental in doses sufficient to produce only a state of drowsiness does not significantly affect the cardiac index. Even during light anesthesia with this drug, there is no apparent effect on cardiac output.[124] However, deep anesthesia with thiopental[124] or a bromide-containing barbiturate*[102] produces a significant decrease in stroke volume (35 to 74 per cent) associated with a somewhat smaller reduction in cardiac output. That the hypotensive response to large doses of barbiturates is, for the most part, due to a reduction in cardiac output rather than peripheral vasodilatation is indicated by the studies of Daniel and associates.[42] Experiments in which the hind leg of the dog was perfused at a constant pressure showed that maximum dilatation was realized at a dose less than one-third that required to produce cardiovascular failure and at a time when the mean blood pressure of the animal was still quite adequate.

The hemodynamic responses to other hypnotics have not been at all as thoroughly studied as those resulting from the barbiturates. In toxic doses, chloral hydrate is stated to produce a marked vasodilatation and hypotension, and its effects on the heart are described as being very similar to those of chloroform.[200] However, no changes in cardiac status have been observed in individuals with normal or dis-

eased hearts who received as much as 2.6 Gm. per day.[4] Cardiovascular studies in man with the more recently introduced hypnotics and sedatives are extremely meager. Methyprylon, when given orally in a dose of 400 mg., has no significant effect on blood pressure or heart rate up to 1½ hours after its administration, neither does it alter the changes in these parameters produced by a change from the supine to the 60 degree, head up position.[132] Although hypotension is known to occur in acute poisoning with the hypnotics, the most striking feature usually is respiratory depression. However, in some cases of acute intoxication with methyprylon[147] and glutethimide,[105, 165] an outstanding manifestation has been a pronounced and persistent reduction of blood pressure. In several instances, this has been out of proportion to the degree of respiratory depression. Whether such a response to these drugs involves primarily a central or peripheral mechanism cannot be stated.

Irregularities in cardiac rhythm have been observed not infrequently with toxic doses of the barbiturates and a number of electrocardiographic changes, e.g., flattened or inverted T waves, depressed S-T segments, high P waves, may occur. The latter have been ascribed to a myocardial oxygen deficit, since not uncommonly they have been noted only after the appearance of cyanosis.[111] Therapeutic doses of certain of these drugs, namely, phenobarbital (120 mg. per day) and methyprylon (400 mg. per day), administered over short periods of time in the presence or absence of chronic cardiac disease produce no significant electrocardiographic alterations.[162] Auricular fibrillation, ventricular premature systoles, and aberrant ventricular conduction have been reported in one instance of poisoning with 12 Gm. of chloral hydrate.[140] Abnormalities produced in the ballistocardiogram by fatigue disappear during sleep induced by the intravenous administration of 250 to 750 mg. of amobarbital.[88] This same drug, when administered to patients with benign hyperten-

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*Narkotal.

sion in amounts (300 to 1000 mg.) sufficient to produce significant hypotension, a ballistocardiographic change, or a disagreeable side reaction, is quite ineffective in improving the ballistocardiogram but effectively improves the calculated maximum force per beat.[158] No significant electrocardiographic changes have been reported in man following administration of therapeutic doses of the newer hypnotic agents (ethchlorvynol, ethinamate, methyprylon, glutethimide).

Autonomic nervous system

All of our more precise information relating to the peripheral autonomic effects of the hypnotics derives from studies on animals other than man. Their significance in the therapeutic applications of these drugs cannot be stated. This being the case, only selected examples of experimental studies of this type will be presented along with the few observations in man which may be related in any way to them.

A number of actions of barbiturates on peripheral autonomic mechanisms have been demonstrated. Marked differences in activity occur with different drugs and different species. In the cat, the intracarotid injection of various barbiturates is without effect on chemoreceptor activity as indicated by impulse activity in the carotid sinus nerve.[118] Pressoreceptor vasomotor reflexes are inhibited in varying degrees by different barbiturates, and, similarly, the cardiac responses to vagal stimulation in dogs and rabbits[125] are affected differently by different drugs in this group. The latter action can be stated to occur at the vagal ganglia since cardiac responses to acetylcholine, physostigmine, and pilocarpine are still elicitable. Transmission in sympathetic as well as parasympathetic ganglia may be impaired by the barbiturates. Amobarbital is about one-fourth as potent as tetraethylammonium in suppressing transmission in the stellate ganglion of the cat,[56] with butabarbital, pentobarbital, hexobarbital, phenobarbital, and thiopental exhibiting de-

creasing activity respectively. There is no correlation between the capacity of these compounds to depress the central nervous system and peripheral ganglionic transmission. Inhibition of transmission in nerve fibers can be demonstrated, but considerably higher concentrations of the drugs are required. In cold-blooded animals, paralysis of autonomic (vagal) nerve endings as well as of ganglia can be produced.[86]

Although serum cholinesterase activity can be inhibited in vitro by various barbiturates, single large doses of phenobarbital or 5-phenyl-5-methylbarbituric acid do not produce significant alterations in the activity of this enzyme in man. However, when the latter drug is administered for a prolonged period of time, the activities of serum cholinesterase in man[166] and spinal cord and muscle (but not brain) cholinesterase in guinea pigs[167] are decreased. Inhibition of cholinesterase could explain the enhanced negative chronotropic response to intravenously administered acetylcholine in the dog during pentobarbital anesthesia.[181] Inasmuch as no effects have been observed in man which are consonant with these findings, it is doubtful that such an enzymatic action of the barbiturates is of any great significance in the interpretation of their human pharmacology. The same undoubtedly is true in the case of the other hypnotics, e.g., chloral hydrate,[48] where an inhibitory action on cholinesterase has been demonstrated.

A number of studies in man and other animals have shown that barbiturates are capable of affecting reflex compensatory mechanisms which are mediated by the autonomic nervous system. The part played by the previously described peripheral actions of these drugs in the alteration of such reflexes is unknown. Dogs deeply anesthetized with pentobarbital exhibit a postural hypotension and do not display the reflex tachycardia which normally follows tilting. In hypertensive patients, the diastolic blood pressure is affected in a quantitatively similar fashion by both seco-

barbital and tetraethylammonium.[71] It is believed that an action on the neurogenic component of the disease is involved in the responses to both drugs but that they act at different levels of the same reflex arc. A decrease in responsiveness of central sympathetic mechanisms is believed to be the explanation for the enhanced hypotensive effect produced by methacholine (Funkenstein test) in hypertensive patients after administration of amobarbital.[163]

Gastrointestinal tract

Although the hypnotics and sedatives, particularly the barbiturates, have been shown to affect the physiologic state of the gastrointestinal tract in experimental animals and these effects are undoubtedly, in many instances, the result of peripheral actions of the drugs, the same cannot be said with respect to man. Both in vivo and in vitro studies have demonstrated the depressant effects of barbiturates on intestinal activity. In dogs the tone of Thiry-Vella loops is decreased by administration of phenobarbital, and the tone of both innervated and denervated stomachs is inhibited by either barbital or pentobarbital. These studies do not explain, however, the relative roles of the intrinsic innervation and the smooth muscle in such responses. The enhanced intestinal activity resulting from administration of barium chloride to dogs can be decreased by certain barbiturates,[192] an effect which again may be attributed to a depressant action on a peripheral cholinergic mechanism or directly on the smooth muscle. A transitory decrease in the contractile amplitude of Thiry-Vella loops in dogs, followed by a gradual recovery with a subsequently greater tone than normal and enhanced contractile amplitude, has been observed during barbiturate anesthesia. Doses (300 mg. orally or 200 to 400 mg. intravenously) of amobarbital sufficient to produce drowsiness or sleep in man cause a reduction of motility in the sigmoid colon.[160] Recovery from the central depressant effects of the barbiturate is associated with manifestations of greater

activity, i.e., enhanced amplitude of contractions and increased tone. Reduction in colonic activity also occurred during various stages of drowsiness and sleep in the absence of drug, and the authors were of the opinion that the reduction of colonic motor activity observed after barbiturate administration was, at least in part, the result of the cerebral inhibition associated with sleep. As in the dog, no explanation was provided for the enhanced activity of the intestine after recovery.

Gastric emptying time can be prolonged and gastric secretions inhibited in animals by the barbiturates, but hypnotic doses in man appear to have little effect on these aspects of gastric physiologic state. The benefits derived from these drugs in allaying symptoms arising from malfunction of the gastrointestinal tract appear to be ascribable, in large part, to their depressant actions on the central nervous system.

Genitourinary system

Depression of tone and contractility of the uterus, ureters, and urinary bladder by barbiturates can be demonstrated in vitro and in laboratory animals in vivo, but the concentrations required usually are not attained after the ordinary therapeutic doses in man. Uterine muscle, in particular, is quite resistant to the depressant effects of these drugs. Parenteral administration of as much as 18 mg. of amobarbital per kilogram over a period of 2 hours during labor has been reported to have no effect on the force, frequency, or duration of uterine contractions.[157] Hypnotic doses do not significantly alter activity, but anesthetic concentrations do prolong labor as the result of a reduction in the frequency and force of contraction. Similar information on the other hypnotics is not available.

Although light barbiturate (pentobarbital) anesthesia in the dog does not significantly affect renal tubular reabsorption of sodium,[169] urine formation can be suppressed by sufficiently large doses as a consequence of (1) hypotension resulting from central vasomotor and peripheral cardio-

vascular depression and (2) enhanced secretion of antidiuretic hormone by an action on the hypothalamic–posterior pituitary system. Certain renal functions in man appear to be more susceptible to alteration by this group of drugs than do those in the dog. Hill, Daeschner, and Moyer[95] determined the effects of sedative doses of phenobarbital and chloral hydrate on several renal functions in children. Phenobarbital had no significant effect on water excretion, but there was an apparent increase after chloral hydrate. Potassium excretion was significantly reduced by phenobarbital but not by chloral hydrate. Glomerular filtration rate and sodium clearance were unaffected, whereas the maximal tubular excretory capacity for p-aminohippurate was reduced by both drugs. Similar reduction of the capacity of the renal tubules to excrete p-aminohippurate has been reported to occur in the dog during surgical anesthesia with barbital or pentobarbital.[76] No significant effects on glomerular filtration rate or effective renal plasma flow were observed. Apparently the state of hydration may affect significantly the influence of barbiturates on renal function since the urinary content of potassium can be reduced by pentobarbital in hydropenic but not hydrated dogs.[198]

Liver

There is no convincing evidence that the commonly employed hypnotics, when given in therapeutic doses to individuals with normal liver function, are hepatotoxic. Of course there are rare instances of hypersensitivity in which liver dysfunction has occurred after administration of relatively small amounts of one of these drugs. This complication has been assumed to have an allergic basis, as in the case of jaundice observed in an individual following the daily administration of ectylurea (900 mg. per day) for a period of 2 weeks.[96] Although it has been stated that jaundice has been observed following ingestion of sufficient amounts of barbiturates to produce acute intoxication and that further hepatic

damage may be produced by these drugs in patients with liver disease, administration of phenobarbital and cyclohexenylethylbarbituric acid to dogs for a year resulted in only minor changes in the morphologic condition of the liver.[11] Single doses (100 to 120 mg.) of pentobarbital administered intravenously to patients with demonstrated liver disease did not significantly alter existing liver function.[170] Removal of the drug from the blood in these patients was not significantly slower than that occurring in normal individuals. The few slight and reversible changes in liver function that have been observed with barbiturate anesthesia and administration of other hypnotics, e.g., 3-methyl-1-pentyn-3-ol carbamate,*[7] may occur following the use of general anesthetics and undoubtedly are a part of the total response of the organism to stress.

Metabolic effects

Metabolic rate and temperature. Anderson, Chen, and Leake[5] determined the effects of a number of barbiturates (amobarbital, barbital, diallylbarbituric acid, probarbital, butethal, cyclobarbital, phenobarbital) on basal metabolic rate. Although their data were not statistically analyzed, gross inspection would indicate no effect of these drugs as long as 1 hour after oral administration of sedative-hypnotic doses. Deep anesthesia in dogs with amobarbital produces a rapid decline in body temperature of 2° to 3° C. and a reduction in basal metabolic rate which, in general, is less than 10 per cent.[44] In the less deeply anesthetized animal, the temperature may drop but shivering can occur with a resultant increase in metabolism and a restoration of the temperature to normal levels. Although barbiturate anesthesia can produce a demonstrable reduction in cerebral oxygen consumption[131] in man and it is stated[80] that total oxygen consumption may be reduced by as much as 20 per cent, well-documented experiments similar to those

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*Oblivon C.

carried out with the dog are not available to support such a statement with respect to either the barbiturates or other hypnotics.

Blood sugar. A number of studies fail to establish any consistent pattern of response to barbiturate administration. The compound and the dose employed, the nature of the diet, and the species are among the factors which determine the magnitude, duration, and direction of any alteration which may occur. In dogs deep anesthesia with amobarbital causes no change in blood sugar levels.[44] However, other work[144] indicates that if the animals are fed a high carbohydrate diet rather than a meat diet, barbiturate administration can cause hypoglycemia. This is an example of the manner in which the response of the blood sugar is affected by diet. It is stated that the usual hypnotic doses of the barbiturates do not significantly or consistently produce changes in the concentration of blood sugar in man, but a variety of abnormal glucose tolerance curves have been reported in patients receiving large doses of these drugs.[135] Such individuals also exhibit higher levels of blood glucose and lactic acid after oral administration of glucose (100 Gm.) than occur in persons receiving no drug.[99]

Other metabolic effects. A variety of biochemical changes have been reported in animals following administration of hypnotics, particularly barbiturates. In the absence of comparable studies and findings in man, it would serve no useful purpose to detail them in a review of the human pharmacology of these drugs. However, it might be of interest to point out certain recent observations[60, 91] on changes in the activities of serum transaminases found in acute barbiturate intoxication in man which have no counterpart in animal studies. In 22 of 37 cases of acute poisoning, serum glutamic-oxalacetic transaminase (GOT) activity was elevated. In 12 of these the value was over 60 U. Ten of the latter complained of muscle pain and tenderness after return to consciousness, and

muscle biopsy revealed necrosis and a reduction of myoglobin. Myoglobinuria was also present. In another series a comparable number showed elevation of GOT activity and only 1 exhibited an increase in glutamic-pyruvic transaminase activity. Those patients who exhibited only an increased serum GOT activity and from whom liver biopsies were obtained showed no evidence of liver cell necrosis.

Miscellaneous effects

The usual therapeutic doses of barbiturates, as well as other hypnotics, produce little if any change in the formed elements of the blood. Thrombocytopenia, hemodilution, a reduction in circulating blood volume, decrease in erythrocytes, cell volume, hemoglobin, and total protein, and other changes of a hematologic nature have been observed following administration of a variety of barbiturates to different animals. In man the occurrence of megaloblastic anemia, responding to administration of folic acid, has been reported after prolonged administration of barbiturates.[33, 36, 65, 173]

The capacity of various hypnotic and sedative drugs to affect skeletal muscle and the myoneural junction has been demonstrated in the laboratory. The various barbiturates can potentiate the maximal twitch response and exhibit curarelike or anticurare activity.[117] Administration of a barbiturate to rats has also been shown to decrease the responsiveness of skeletal muscle to direct electrical stimulation.[180] The presence and amount of activity of these various types depend on the particular compound employed. Perhaps some of them may be observed in man after large toxic doses of the barbiturates, but it is doubtful that they are of any significance in the therapeutic use of these drugs. The bromide ion,[93] paraldehyde,[143] and chloral hydrate[178] can alter the functional state of skeletal muscle or the myoneural junction, but analogous actions in man have not been reported.

Local irritation of the skin or mucous

membrane is not an uncommon property of the hypnotic drugs. The extreme alkalinity of salts of the barbiturates is well known, and the consequences of depositing strong solutions of these in certain sites, e.g., beneath the skin, are recognized. Occasionally, marked irritation with edema of the mucous membranes of the throat can occur following ingestion of barbiturates.[123] Nausea and vomiting are not rare after oral administration of most hypnotics. Large amounts of sodium or potassium bromide not infrequently produce gastric irritation, presumably on the basis of a salt effect. Chloral hydrate, when applied to the skin, is capable of producing inflammatory changes, and this irritant action is believed to be the basis of the nausea and vomiting which may follow ingestion of insufficiently diluted solutions of the drug. Old decomposed preparations of paraldehyde containing significant concentrations of acetic acid have caused marked irritation and necrosis of mucous membranes after oral or rectal administration,[2] and at least two deaths have been ascribed to complications arising from such effects.

Other than the cutaneous alterations which can be produced by direct application of the more irritant hypnotics, undoubtedly the untoward reactions which have been observed in the skin are idiosyncratic or have an allergic basis. A variety have been described and their occurrence is unpredictable. In the case of the barbiturates, these range from a generalized morbilliform rash, bullous erythema multiforme, discrete coin-sized violaceous macules of "fixed-eruption" type, and urticaria to universal exfoliative dermatitis. They infrequently occur following a single dose but appear to result from an acquired sensitivity. Cutaneous changes have been reported to occur in 4 per cent of individuals acutely intoxicated with the barbiturates,[97] and although most cases of barbiturate dermatitis clear on withdrawal of the drug, death has followed such eruptions.[177] In chronic bromide intoxication, an acneform dermatitis is seen in about 20 per

cent of the individuals, but the eruption may be more bizarre, e.g., nodose bromoderma. The involvement of the skin is unrelated to other manifestations of intoxication or blood levels of bromide. A variety of allergic cutaneous reactions, as well as somnambulistic reactions, may occur after administration of chloral hydrate.[37] Acute exfoliative dermatitis after methylparafynol has been observed, and it is likely that cutaneous eruptions have been or will be noted with the newer agents.

Tolerance and summation or synergism

Development of tolerance or cross-tolerance[77, 86] to various barbiturates has been demonstrated in laboratory animals. Relatively large doses (40 to 50 per cent of the LD_{50}) were employed, and tolerance was relative (manifested by a shortening of the sleeping time without any change in the LD_{50}) and rapidly lost. Belleville and Fraser[10] undertook a study to determine the degree of tolerance developed to barbiturates in man. In their investigation, tolerance was defined as a decrease in the initial degree of observable effects when the same dose of drug was administered repeatedly. Eighteen former addicts were given 0.4 Gm. of pentobarbital or secobarbital daily for 90 days and observed clinically. Psychomotor tests (reaction time, vertical tracing) were administered before and during drug treatment as well as after withdrawal of the drug. A significant degree of tolerance occurred to all the initial effects of the barbiturates, as indicated by hours of sleep, intoxication scores, and psychomotor test performance. Evidence of tolerance appeared early, during the first week or two of intoxication. There was marked individual variation in the degree of tolerance developed,[69] and in most individuals a maximum daily dose could be established which, if exceeded by as little as 0.1 Gm., resulted in serious intoxication. Evidence of tolerance to the electroencephalographic changes produced by barbiturates also has been reported,[52] but only when somewhat higher doses (0.6

Gm. per day) of secobarbital or pentobarbital were employed. Individuals previously exposed to barbiturates and acutely intoxicated with phenobarbital may exhibit blood levels of barbiturate one and one-half to two times those of individuals having no prior exposure to the drug without evidencing any greater degree of depression.[179] The former were stated to be rational when they experienced blood levels of 7.5 mg. per cent, at which concentration the normal person would be anesthetized.

In laboratory animals a limited degree of tolerance to chloral hydrate[193] and paraldehyde[35] has been demonstrated after extended administration of large doses. No tolerance to the depressant effects of the bromide ion has been observed, and data on this point in the case of the newer agents are not available. Likewise, experiments designed to assess the capacity of man to develop tolerance to these hypnotics have not been performed.

The realization of a greater degree of depression from administration of a second depressant of the central nervous system along with a hypnotic than is obtained with the hypnotic alone is recognized and requires no documentation. Whether the resultant degree of the response obtained by combination of hypnotics with other agents can be ascribed to summative, synergistic, or potentiating actions is difficult, if not impossible, to state in any given situation at this time. Quantitative data in man which would allow one to provide some enlightenment in this area are almost totally lacking. There are many agents (calcium salts, acetylcholine, disulfiram, certain surface active agents, mephenesin, a number of intermediary metabolites, thiamine) which would appear to give rise to other than an additive effect when used in combination with the barbiturates.[156] Here again, however, it is not possible to draw any conclusions with respect to the quantitative nature of the drug interactions. The nearest one can arrive at a positive statement with regard to these relationships derives from studies[62] on the

combination of alcohol with a barbiturate. Whereas a definitely additive effect is demonstrable on the basis of one type of measured response in mice, the picture appears to be much more complex in man[103] (see *central nervous system, behavioral effects*).

Addiction

It has been pointed out by Fraser[68] that the Germans recognized the addiction liability of the barbiturates at a much earlier date than did physicians and medical scientists in this country. However, it was the careful studies on animals[168] and man[68] in the United States which provided the detailed information on addiction to these compounds and a basis in fact for the earlier clinical observations. A description of the various aspects of human addiction has derived largely from the efforts of the Research Division, U. S. Public Health Service Hospital, Lexington, Kentucky, and the work prior to 1957 is excellently summarized by Fraser.[68] Subsequent additional information is contained in publications by Fraser and colleagues[70] and Essig and Fraser.[52]

That the symptoms and signs of abstinence from the barbiturates are not attributable to an antecedent epileptic or psychotic diathesis was established early in the course of studies on man. Subsequently, it was demonstrated that continuous ingestion of barbiturates over a long period of time resulted in physical dependence. The large doses required to result in tolerance and physical dependence to these compounds distinguishes them from the opiates, of which only small doses are needed to initiate the changes that result in these phenomena. It is this quantitative difference which allows the use of therapeutic doses of the barbiturates for years without establishment of addiction. The intoxication produced by chronic administration of the barbiturates resembles that of chronic alcoholism, and the abstinence syndrome is distinctly different from that following withdrawal of opiates. The degree

of physical dependence developed depends on a number of factors, among which are (1) the barbiturate employed, (2) frequency of dosage, (3) total daily dosage, (4) length of administration, (5) age, (6) sex, (7) race, and (8) personality. These are among those several factors which affect the response to any drug and are in no way unique. The only one of these variables to which any definition can be given is dosage. With secobarbital or pentobarbital, a daily dose in excess of 0.4 Gm. in healthy adult males is required to produce a clinically significant degree of physical dependence. Below this dose, i.e., 0.2 Gm. per day, administration for a period of 1 year results in no signs of abstinence upon withdrawal of the drug. Following daily administration of 0.4 Gm. for 90 days, minor symptoms and signs occur, and above this dose, i.e., 0.6 to 0.8 Gm. per day, persistent, mild intoxication is experienced. As stated previously, there are a marked individual variation in the degree of tolerance developed to these drugs and a critical level of tolerated dosage which cannot be significantly exceeded without danger of severe intoxication.

During the period of intoxication with doses sufficient to result in physical dependence, a number of signs and symptoms occur. These include impairment of mental ability, regression, confusion, emotional instability, nystagmus, dysarthria, ataxia, and depression of superficial and abdominal reflexes. A certain degree of tolerance to these effects develops. Upon withdrawal of the drug, certain symptoms and signs occur which have been classified as major or minor in nature. The minor symptoms and signs include, in approximate order of appearance, anxiety, involuntary twitching of muscles, coarse intention tremors of the hands and fingers, progressive weakness, dizziness, distortions in visual perception, nausea, vomiting, insomnia, weight loss, and marked orthostatic hypotension. Convulsions of the grand mal type and a delirium resembling alcoholic delirium tremens are the major manifestations of withdrawal.

When the drug is withheld, signs and symptoms of intoxication gradually disappear during the first 8 to 12 hours, and between 8 and 36 hours the minor evidences of withdrawal appear. These then decrease in severity unless there are convulsions and/or delirium, which then are followed by tremor, anxiety, and insomnia persisting for 8 to 14 days. Rather marked electroencephalographic changes consisting of either high voltage paroxysmal discharges or high voltage paroxysmal activity of a slow type occur. Following a convulsion, certain changes occur in the blood which are like those found after electroshock. There is a sharp increase in serum uric acid concentration, a subsequent decrease in the eosinophil count, and a delayed rise in the concentration of nonprotein nitrogen. Death has occurred during withdrawal[69] and was characterized by a rapid weak pulse, cyanosis, continuous clonic movements, hypotension, and hyperthermia.

In the older literature, addiction to chloral hydrate and paraldehyde has been reported, and it does not seem unlikely that this may occur under appropriate circumstances, but experimental studies of the type employed in the case of the barbiturates have not been undertaken to confirm such observations. At least 2 cases of what appear to have been addiction to glutethimide have been reported in the United States.[126, 159] In 1 case the patient was known to have taken the drug for 7 months and to have ingested at least 50 Gm. during the final 10 days prior to being seen by a physician. During withdrawal the patient was confused and disoriented and convulsions of the grand mal type were observed. In the second case it was not possible to establish the amount of drug being taken, but it was estimated 0.5 Gm. had been used two or three times a day over a period of 9 months. Although no convulsions occurred on abrupt withdrawal, the patient was extremely restless, exhibited periodic muscular spasms, and on the sixth day experienced hallucinations although receiving

phenobarbital. Other reports of a similar nature will undoubtedly appear in the literature.

Disposition

Absorption. Since the hypnotics are effective when administered by a variety of routes, it would follow that their absorption from most enteral and parenteral sites is quite adequate. Only a limited amount of information is available on the quantitative aspects and kinetics of absorption of these drugs in man. Absorption of significant amounts of both thiopental and secobarbital from the human stomach has been shown to occur. Lous[128] has examined the relative rates of absorption of three barbiturates from the intestinal tract by following their concentrations in blood after oral administration. Barbital and aprobarbital appeared to be absorbed at about the same rates, as estimated by the time required to attain maximal plasma levels, 4 to 8 hours and 3 to 9 hours, respectively. The highest concentrations of phenobarbital in plasma were realized after a significantly longer time, 12 to 18 hours. Both the clinical and laboratory data of Graham[82] indicate alcohol does not significantly influence the rate of absorption of barbiturates from the gastrointestinal tract.

The bromide ion is rapidly absorbed from the gastrointestinal tract, and studies employing isolated segments of intestine have shown that although the chloride ion does not interfere with absorption of bromide, rate of absorption of chlorides is reduced in the presence of the bromide ion.[17] The lower portions of the intestine are stated to absorb bromides more rapidly than are the upper portions. Maximal plasma levels of methyprylon in the dog are achieved approximately 6 hours after its oral administration.[152] The absorption of ethinamate is rapid, since it appears to be complete in the dog in 30 minutes.[119]

Distribution. Most of the data obtained prior to 1956 on the distribution (and metabolism) of barbiturates have been well summarized by Richards and Taylor.[156] Of

necessity, most of our information on this aspect of the pharmacology of these drugs derives from studies on species other than man. These indicate that the barbiturates gain access to practically all tissues, the concentrations found being determined by the type of compound, dose, and time following administration. After finding their way into the blood, they are bound to plasma protein, the albumin fraction, to a varying degree. The extent to which binding occurs will determine the relative amount of freely diffusible drug available at any given time for distribution to other tissues. It is this latter fraction which determines the magnitude of the physiologic effect. Among the several barbiturates studied, barbital is bound in the smallest (5 per cent) and thiopental and hexethal (65 per cent) in the greatest proportions. Binding is reversible and dependent on concentration of albumin. In general, the degree of binding is inversely proportional to duration of action. The effect of pH on binding is not great, but in vivo plasma concentrations of phenobarbital vary in the same direction as pH.[24] Tissue/plasma concentration ratios of phenobarbital vary in a direction opposite to those of pH, such changes being due to alterations in distribution of the drug between plasma and tissue. The influence of pH on the distribution of barbital is not at all as marked as it is in the case of phenobarbital, which is explained by the difference in acidic strength of the two compounds.

With few exceptions, distribution of barbiturates from blood to other tissues and establishment of equilibrium occur quite rapidly. Barbital and phenobarbital enter the brain more slowly than other compounds which have been studied. This would explain the delay in onset of anesthesia after their intravenous administration. The ultra short–acting barbiturates, e.g., hexobarbital, thiobarbiturates, pass very slowly from blood into fat but attain very high concentrations in adipose tissue. This aspect of the distribution of these compounds is of significance in relation to

their short duration of action. The concentration ratios of barbiturates in brain and blood approximate unity, but tissue/blood concentrations are higher for kidney and liver, which is undoubtedly ascribable to the greater degree of protein binding which is known to occur in these tissues. There is no significant difference in distribution to the various areas of the brain.

Concentrations of thiopental in cerebrospinal fluid of the dog at equilibrium are about 75 per cent of those found in plasma water.[24] Concentrations of other barbiturates in the cerebrospinal fluid have been stated to be usually very low,[2] but in man Lous[129] has found the ratio of concentration of phenobarbital in cerebrospinal fluid to that in serum to be 0.43 to 0.60. These values are based on concentrations of phenobarbital in serum. Those experiments in which concentrations of phenobarbital were determined in ultrafiltrates of serum show the ratios to approximate unity.

Barbital,[66] phenobarbital, amobarbital,[150] pentobarbital,[61] and the thiobarbiturate buthalitone[104] readily cross the placental barrier and are found in fetal tissues. Concentrations of pentobarbital are approximately 75 per cent of those found in maternal blood. Equilibrium between the two appears to be established within 30 minutes after administration of amobarbital. Both amobarbital and phenobarbital are found in high concentration in the placenta and fetal liver and brain. Small amounts of barbiturates find their way into intestinal secretions and have been detected in the skin and hair of guinea pigs. It has been stated that the permeability of human erythrocytes to barbiturates is extremely low, but this is doubtful since concentrations of barbiturates in plasma and whole blood of rabbits are approximately equal.

The bromide ion is distributed in the body in a manner similar to the chloride ion, and the bromide space in man is roughly the same as that for chloride.[92] Bromides remain extracellular except in the case of the erythrocyte[145] and perhaps the cells of the central nervous system.[20] Equilibrium between human plasma and erythrocytes in vitro occurs rapidly, and the ratio plasma/erythrocytes approximates but is slightly less than 1.[98] Except for brain, the bromide/chloride ratio in the extracellular fluid in cats and dogs is the same as that for serum.[194] In man at least 24 hours is required for the establishment of equilibrium between plasma and cerebrospinal fluid,[98] and the ratio cerebrospinal fluid bromide/serum bromide, at equilibrium, is approximately 0.4.[39] This ratio is essentially the same in a number of mental disorders, schizophrenia, affective disorders, senile dementia, puerperal psychosis, and general paresis. However, in inflammatory neurologic diseases, the rate of entry of bromide into cerebrospinal fluid is considerably more rapid than it is in the absence of inflammation.[18] A preferential distribution to certain areas of the central nervous system is suggested by studies with Br[82] in the cat.[20] Higher concentrations are attained more rapidly in the hypothalamus than in parietal cortex, white matter of cerebellar hemispheres, medulla, or spinal cord. Differences in permeability of these regions to the bromide ion are suggested as an explanation for such findings.

Little is known concerning the distribution of chloral hydrate in the body. It and/or its metabolites undoubtedly can be found in most tissues, and it does cross the placental barrier. Bernstine, Meyer, and Hayman[14] reported that shortly after administration of chloral hydrate to pregnant women at term, the drug is found in fetal blood, where significant levels are rapidly attained. Trichloroethanol is present in amniotic fluid more consistently than the parent compound or the metabolite, trichloroacetic acid, the levels of trichloroethanol being comparable to those found in fetal blood. Paraldehyde also traverses the placenta and is found in umbilical cord blood in concentrations approximating those in maternal blood.[73] Amounts of paraldehyde sufficient to produce fatal intoxication result in a blood/brain ratio of 1.27.[63] Methylparafynol has been detected

in a number of body fluids and tissues. It is present in blood and milk within 90 minutes after ingestion of 2 Gm., can be found in amniotic fluid after oral administration of 1 Gm. to the mother, and is recoverable from fetal liver and heart.[6] After intravenous infusion of 1.1 Gm., it was detected in cerebrospinal fluid and its volume distribution ranged from 39 to 44 per cent. Data from studies on laboratory animals indicate glutethimide is widely distributed throughout the body. Although McBay and Katsas[130] were unable to detect any glutethimide in blood or liver from 4 individuals dying as the result of an overdose of the drug, Griffon, LeBreton, and Janvier[84] reported blood concentrations of 56 and 110 mg. per liter in 2 fatal cases of poisoning. They also found concentrations of the drug in the kidneys and liver two to four times those in the blood.

Metabolism. Although the rate of disappearance of drugs from the blood usually is a reflection of distribution and the various rates of metabolism and excretion, it can be stated there is a rough inverse relationship between the rate of removal of the barbiturates and their duration of action. In man Richards and Taylor[156] have attempted to approximate the rate of decline of blood concentrations for various barbituric acid derivatives on the basis of data available in the literature. Long-acting compounds, e.g., barbital and phenobarbital, are removed at the rate of about 20 per cent per day or, if one extrapolates, approximately 0.8 per cent per hour. Intermediate to short-acting barbiturates, e.g., aprobarbital and pentobarbital, disappear at the rate of 2 to 4 per cent per hour. Blood levels of ultra short–acting compounds, e.g., hexobarbital and thiobarbiturates, decline about 10 per cent per hour. These values certainly are not mathematically correct since they assume only first order kinetics is involved, but they do provide useful estimates.

The barbiturates have been shown to undergo a number of chemical changes in the organism which have been classified under four general headings by Raventós[154]: (1) oxidation of radicals in the 5 position, (2) removal of N-alkyl radicals (3) conversion of thiobarbiturates to their oxygen analogues, and (4) hydrolytic cleavage of the barbituric acid ring. The last appears to occur only to a minor extent in vivo but has been demonstrated in the dog for pentobarbital and amobarbital. Oxidation of groups in the 5 position appears to be the most important mechanism for metabolic transformation in man, although most studies of this type have been done in other species. Phenobarbital is converted to 5-ethyl-5-(p-hydroxyphenyl) barbituric acid, a portion of which is excreted in the conjugated form, presumably as the sulfate.[51] The diastereoisomers of 5-ethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid are found in the urine of man after administration of pentobarbital.[134] The thiobarbiturates also undergo oxidation of radicals in the 5 position, e.g., oxidation of thiopental to 5-ethyl-5-(3-carboxy-1-methylpropyl) thiobarbituric acid.[204] Little or no information is available to indicate the extent to which the parent compounds are converted to these oxidation products or the role such metabolic alteration plays in the disposition of other barbiturates which have been similarly studied in man. Conversion of mephobarbital to phenobarbital is an example of the N-dealkylating mechanism in man. Butler[30] has demonstrated the presence of this metabolite in the plasma of individuals receiving mephobarbital and found its concentration there to be more than three times that of the parent drug. For a discussion of the enzymatic mechanisms involved in the previously discussed metabolic alterations as well as conversion of thiobarbiturates to their oxygen analogues, a reaction which to date has not been demonstrated in man, the reader is referred to the review by Brodie, Gillette, and LaDu.[25]

Urochloralic acid was isolated from the urine of patients receiving chloral hydrate in 1875, and it was identified as the glucuronide of trichloroethanol in 1882. In

1948 Butler[29] showed chloral hydrate was converted to both trichloroethanol and trichloroacetic acid in the dog, and subsequent studies by Marshall and Owens[133] demonstrated the occurrence of the same metabolic alterations in man. The amount of chloral hydrate oxidized to trichloroacetic acid varies considerably in different individuals and from day to day, ranging between 5 and 47 per cent of the administered dose. Relatively high plasma concentrations of trichloroacetic acid coincide with the appearance of trichloroethanol. In general, the concentration of the latter is less than the former. Apparently, the major portion of a dose of chloral hydrate is converted to trichloroacetic acid and only small amounts of the glucuronide of trichloroethanol are found in the blood stream.

Very little can be said regarding the metabolism of paraldehyde by man. Since it is capable of furnishing a two carbon fragment for acetylation of sulfanilamide, this suggests that after depolymerization to acetaldehyde and oxidation of the latter to acetic acid, it is further metabolized to carbon dioxide and water. Acid products of its oxidation, under certain circumstances, can result in metabolic acidosis.[51, 90, 195] Like paraldehyde, methylparafynol appears to be almost completely metabolized, no unaltered drug being found in the urine after an oral dose of 100 mg. and no more than 15 per cent appearing after doses of 500 to 3,000 mg.[148] In vitro experiments utilizing liver and kidney tissues have shown the drug can be completely metabolized to carbon dioxide and water. In the dog, small amounts (3 per cent) of methyprylon are excreted as the dehydrogenated derivative, 2,4-dioxo-3,3-diethyl-5-methyl-1,2,3,4-tetrahydropyridine.[152] The formation of this derivative by man has not been described. In fatal poisoning with glutethimide, both the free and conjugated metabolite, α-phenylglutarimide, a de-ethylated derivative of the parent compound, have been found in the urine, the former present to the extent of about 10 to 30 per cent of the latter.[130] Approximately 1.5 per cent of the administered dose of glutethimide in the dog is excreted as α-phenylglutarimide.[172]

Experimental studies have adequately demonstrated the major role played by the liver in the metabolism of those barbiturates whose actions are limited by such a means, but the picture is not as clear cut in man. Apparently a significant amount of liver dysfunction is essential before any appreciable alteration in the duration of action of these compounds is apparent.[47, 174] When hypnotic doses (100 to 120 mg.) of phenobarbital were given to patients with severe liver disease, the drug was not found to disappear less rapidly from the blood than it did from individuals with normal liver function.[170] However, it is doubtful that these were sufficiently challenging doses of a compound which is excreted unchanged in significant amounts to result in any observable differences in rates of metabolism in the two types of patients studied. Tissues other than the liver have been demonstrated to be capable of metabolizing the barbiturates, but they would appear to play a much less important role.[156]

The liver as a site of numerous enzymatic mechanisms for metabolism of drugs becomes apparent on reading the review by Brodie, Gillette, and LaDu,[25] and it would be safe to say, without any experimental data, that it plays a significant role in the chemical alteration of all metabolizable hypnotics. This has been borne out by studies which have been performed with laboratory animals. Reduced liver function, produced by various means, prolongs the action or slows the rate of metabolism of chloral hydrate, paraldehyde, and ethinamate.[46] These and some of the other hypnotics also have been shown to be metabolized in vitro by preparations of hepatic tissue.

Excretion. Renal excretion is the major mechanism for elimination of barbiturates from the body. Barbital is the only member of the group which owes its long duration

of action almost entirely to its slow rate of excretion. Up to 90 per cent of a dose may be excreted in unaltered form.[46] In the dog the renal clearance of barbital is about one-tenth of glomerular filtration rate. This is believed to be the result of its reabsorption in the tubules as a free acid by a process of back diffusion.[75] In man, simultaneously determined urea and barbital clearances have been stated to exhibit good correlation.[26] Clearance values for barbital have been found to range from 4 to 40 ml. per minute in individuals with varying degrees of kidney insufficiency. This is approximately 0.03 to 0.3 the average value for glomerular filtration rate. Phenobarbital, diallylbarbituric acid, and aprobarbital are excreted in an unaltered form to the extent of 30 per cent of the total administered dose. Only small amounts of the other barbiturates find their way into the urine without undergoing chemical changes. Renal clearance rates for phenobarbital and aprobarbital, like barbital, are very low. Values reported for phenobarbital are 0.7 to 4.5[128] and 1 to 9[26] ml. per minute, and aprobarbital is cleared at the rate of 1 to 8 ml. per minute.[128] These compounds, like barbital, can be detected in the urine for several days after administration of a single dose. The different values reported for clearance of phenobarbital could be due to the rather pronounced influence of urinary flow and pH on its rate of excretion. Clearance increases with an increase in urine flow and, at any given rate of urine flow, the clearance is much higher when the urine is alkaline than when it is acid.[24] If it is assumed the drug is reabsorbed by passive back diffusion and the renal tubules are permeable to the undissociated but not the ionic form, these observations become understandable. By administration of alkali to patients acutely intoxicated with phenobarbital, the rate of excretion of the drug has been enhanced.[139] Elimination of barbiturates by other than the renal route is relatively unimportant. Small amounts may be found in the milk,[161] and traces find their way into

the feces and perspiration. In experimental animals, they also have been detected in hair as much as 1 month after administration.

Excretion of the bromide ion proceeds by the same routes as the chloride ion, renal elimination being the most important. Its slow excretion by this channel is attested to by the fact that traces of bromide can be detected in the urine as long as 20 days after a single dose. Renal elimination of bromide is somewhat slower than that of chloride. The experiments of Wolf and Eadie[202] on the dog suggest that the total halide reabsorbed, rather than chloride or bromide separately, is related to the water reabsorbed. They believe the ratio of bromide to chloride reabsorbed is determined by the ratio of bromide to chloride filtered by the glomerulus and exceeds it except when there is almost complete reabsorption of the two ions. The fraction of bromide reabsorbed is a function of the fraction of chloride reabsorbed, the absolute amount of chloride reabsorbed, and the amount of bromide filtered. Rate of excretion of bromides is enhanced by administration of sodium chloride and is stated to be further increased by ammonium chloride.[40] Other studies[100] indicate the mercurial diuretics enhance the effectiveness of chloride administration but that ammonium chloride is less effective than sodium chloride. In the dog, as much as one-fifth of an ingested dose of an inorganic bromide will be excreted in the feces after prolonged administration of a bromide salt.[16] The presence of hydrobromic acid has been demonstrated in the gastric secretions of individuals receiving bromides[151] when the concentration of bromide may exceed that in blood. Other less significant channels of exit for the bromide ion are perspiration, tears, milk, saliva, etc.

The presence of the glucuronide of trichloroethanol, trichloroethyl-β-glucuronide, in the urine has been referred to previously. Chloral hydrate also is excreted as free trichloroethanol and trichloroacetic acid. Marshall and Owens[133] found the

renal clearance of trichloroacetic acid to vary considerably from individual to individual, ranging from 5 to 39 L. per day, values considerably below those for filtration rate. An average of 4.6 (range 0.5 to 19) per cent of a dose of chloral hydrate was recoverable from the urine as free trichloroethanol. The total trichloroethanol excreted varied from 16 to 35 per cent of the dose. Their data indicate that the major portion of chloral hydrate is found in the urine as trichloroacetic acid. Chloral hydrate also has been found in milk.[161]

The excretion of paraldehyde by the pulmonary route is common knowledge to anyone who has associated in any way with an individual who has ingested the drug. Most of our information on elimination of this hypnotic, however, derives from experiments on animals. In the dog,[122] pulmonary excretion accounts for 11 to 28 per cent of an administered dose, and 2.5 per cent or less is excreted by way of the kidneys. After a small dose (100 mg.) of methylparafynol in man, no free drug is detectable in the urine, but after larger amounts (0.5 to 3 Gm.), as much as 14.2 per cent is excreted in unaltered form.[148] McBay and Katsas[130] have found both the free and conjugated metabolite (α-phenylglutarimide) of glutethimide in the urine of individuals dying from an overdose of this drug. Kier, Whitehead, and White[110] state that the rate of renal excretion of glutethimide in acute poisoning is very slow and constant (2 to 3 mg. per hour).

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Clinical pharmacology of antiarrhythmic drugs

Important advances in the pharmacology and clinical use of antiarrhythmic drugs have resulted recently from improved methods of diagnosis, from increased knowledge of alterations of cardiac metabolism and the effect of electrolyte disturbance on arrhythmias, and from increased knowledge of the pharmacology of old and new antiarrhythmic drugs. These are discussed in detail.

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The drug therapy of cardiac arrhythmias is complicated by many factors: (1) There are many types of arrhythmias, ranging from those caused by disturbances in the atrium, in the A-V node, and the bundle of His to those that arise in the ventricle; the response to therapy varies considerably, depending on the site of the ectopic focus. In addition, more than one disturbance of cardiac rhythm may operate simultaneously. (2) The underlying cause varies widely—infection, degenerative states, congestive failure, anoxia, electrolyte imbalance, or purely functional conditions; often a combination of factors is operative. (3) Some antiarrhythmic drugs exert more than one action.

Although the number of drugs available has increased over the years, the list of dependable preparations is still relatively small. In a general way, drugs can influence the cardiac rhythm by increasing the sympathetic activity to the heart (sympathomimetics), by reducing it (sympathetic blockade), by increasing parasympathetic activity (parasympathomimetics), and by decreasing it (parasympathetic block). Some drugs exert two types of actions: (1) decreased sympathetic and parasympathetic activity (ganglion block) and (2) increased sympathetic and parasympathetic activity (some sympathomimetic drugs, e.g., norepinephrine). Other drugs have an effect that is independent of cardiac innervation (electrolytes, alkalinizing agents), and still others have both a direct action on the heart muscle and some action on the parasympathetic innervation (quinidine, procaine amide, digitalis).

This article will group drugs according to their primary antiarrhythmic action, and each drug will be discussed in terms of mechanism of action, administration, dosage, and untoward and toxic effects.

Sympathomimetic drugs that increase cardiac rhythmicity

From the standpoint of effect on cardiac rhythm, sympathomimetic drugs increase cardiac rhythmicity. However, these agents

may also decrease rhythmicity through a vagal reflex.

Epinephrine. This sympathomimetic amine is indicated for increasing pacemaker activity and for vasopressor effect in the following conditions: periods of cardiac arrest, episodes of marked slowing of heart rate, especially if accompanied by hypotensive states, Stokes-Adams seizures, and cardiac complications of anesthesia and surgical procedure. With the development of acidosis, epinephrine and other sympathomimetic drugs exert less vasopressor action and have decreased effect on cardiac rhythmicity. This may occur during anoxia and shocklike states incident to episodes of cardiac arrest. Reduction in acidosis or restoration of acid-base balance reverses these effects.[32, 59]

Mechanism of action. The effect of sympathomimetic amines on atrial muscle is much less dramatic than on pacemaker tissues. The effect of epinephrine on the action potential in atria is not often impressive,[18, 40] but its effect on ventricular muscle is noteworthy. An increase in epinephrine dosage sufficient to increase frequency of contraction will also shorten the duration of the action potential. This is the cause of ventricular fibrillation which sometimes complicates the administration of epinephrine.[18]

Subcutaneous injection of epinephrine causes a rise in pulse rate, systolic blood pressure, and cardiac output and a reduction in diastolic pressure presumably resulting from vasodilatation. Epinephrine also directly increases myocardial irritability, shortens the refractory period of atrial muscle, and accelerates atrioventricular conduction. Coronary blood flow is increased, but at the expense of increased cardiac work.

Administration. During the cardiac arrest of Stokes-Adams seizures (or that associated with other conditions), epinephrine may be administered by intracardiac injection (0.25 to 1 ml. of a 1:1,000 solution). To maintain an adequate heart rate (30 to 40 beats per minute) and to prevent further

seizures (particularly during a state of hypotension or shock), 0.2 to 0.3 ml. of a 1:1,000 solution (diluted tenfold) of epinephrine should be given intravenously by slow drip under close observation.[6] Intravenous injection of epinephrine should be avoided, however, when subcutaneous or intramuscular injection is sufficient. When the indications are less urgent, epinephrine may be given in doses of 0.2 to 0.3 ml., subcutaneously, every 1 to 2 hours, or it may be given as a 1:500 solution in oil (1.0 ml. intramuscularly).[6]

The effect of subcutaneous injections of 0.25 to 1.00 ml. of a 1:1,000 solution of epinephrine has been studied in patients with complete heart block during and in the absence of Stokes-Adams seizures.[29, 47, 63] The onset of action is rapid (2 to 4 minutes). Ventricular acceleration is directly related to the rate immediately before the injection; high initial rates are followed by little or no gain in rate, slow rates by pronounced acceleration. Usually 0.25 ml. of epinephrine (1:1,000) will produce as much acceleration as a much larger dose; it is therefore a ceiling dose. As a general rule, the peak reaction of the atria develops before the ventricles have completed acceleration. Cardiac acceleration persists after the blood pressure has returned to its previous level. Occasionally, epinephrine may temporarily restore a sinus pacemaker.

The effect of sympathomimetic amines was studied in 21 patients with Stokes-Adams seizures during complete absence of ventricular activity.[63] Excitation of ventricular pacemakers under these circumstances was used as the criterion of efficacy of the drugs. Epinephrine administered intravenously was very effective in initiating, maintaining, and accelerating an idioventricular pacemaker during induced Stokes-Adams seizures.

Infusion of epinephrine may also precipitate ventricular flutter or fibrillation. This is most important, since the underlying disturbance may be the prefibrillatory type of ventricular tachycardia or ventricular flut-

ter or fibrillation (not cardiac standstill) which may be reversible and proceed to spontaneous recovery, whereas epinephrine administered at this time may precipitate irreversible ventricular fibrillation.[29, 47, 63]

Untoward and toxic effects. Epinephrine may induce irreversible ventricular tachycardia or ventricular fibrillation. Constant electrocardiographic monitoring during infusion is therefore mandatory. Cerebrovascular accidents and coronary occlusion have occurred following the intravenous and subcutaneous injections of epinephrine.

Isoproterenol. Isoproterenol* is a congener of epinephrine in which the methyl group attached to the nitrogen is replaced by an isopropyl group. This change decreases the excitatory sympathomimetic activity of isoproterenol (on smooth muscle) and increases its inhibitory action, i.e., vasodilatation and bronchodilatation. Unlike epinephrine, therefore, isoproterenol has no vasoconstrictor action, but it exerts a direct action on the myocardium.

Isoproterenol is useful for increasing cardiac rhythmicity during Stokes-Adams seizures.[8, 47, 63] It occasionally has effectively increased the rhythmicity of the ventricular pacemaker and restored adequate cardiac output, even though infused during Stokes-Adams seizures with numerous ventricular extrasystoles or ventricular flutter or fibrillation.[8, 53, 63] The chronotropic activity of isoproterenol decreases in the presence of acidosis, as is the case with epinephrine, but this change is reversible.

Mechanism of action. The action of isoproterenol on the heart resembles that of epinephrine, but its positive chronotropic and inotropic actions are more marked. It differs from epinephrine in that its activity is largely restricted to the higher cardiac pacemakers (sinus and A-V nodes). It is about 5 times as potent as epinephrine in stimulating cardiac pacemakers.[51] Epinephrine tends to produce ventricular fibrillation, particularly in the presence of previous cardiac damage. Under similar

conditions, isoproterenol does not usually produce this arrhythmia. It increases the ventricular rate in bradycardia and may stimulate the ventricle to take over during episodes of asystole. It may also restore pacemaker activity during Stokes-Adams seizures, even in the presence of transient periods of ventricular tachycardia or ventricular fibrillation. Greater potency in these respects and the absence of pressor action generally make isoproterenol preferable to epinephrine in the therapy and prevention of cardiac arrhythmias and cardiac standstill.

Administration. The drug may be administered sublingually in doses of 10 to 20 mg. every 2 to 3 hours; subcutaneously, 0.2 mg. every 6 hours; intravenously as a continuous infusion of 1 mg. of isoproterenol dissolved in 200 ml. of 5 per cent glucose in distilled water, or 5 μg per 1 ml. at a rate of 9 to 200 drops per minute.[63]

Untoward and toxic effects. Few untoward effects are observed with sublingual administration. However, isoproterenol should be infused with caution in acute coronary occlusion or heart failure. In patients with heart disease, it may induce ventricular tachycardia or ventricular fibrillation at times although such an occurrence is much less likely than with epinephrine. Other undesirable effects include nausea, headache, nervousness, tremor, dizziness, precordial or anginal pain, weakness, and sweating.

Ephedrine. Ephedrine is indicated for long-term administration to increase cardiac rhythmicity. It is of value in preventing the Stokes-Adams seizures which may occur with cardioinhibition in the carotid sinus syndrome as well as seizures associated with complete A-V heart block. Ephedrine may also be helpful for prevention of the episodes of hypotension which are associated with these attacks.

Mechanism of action. Ephedrine raises the blood pressure, stimulates the heart muscle, accelerates the heart rate, and increases the metabolic rate. Compared to epinephrine, the onset of the pressor re-

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sponse is slower and the resultant vasopressor effect is much more sustained.

Administration. Ephedrine is administered orally in a dose of 15 to 30 mg., four to five times per day. It is often combined with sedatives which counteract the nervous symptoms produced by it. Ephedrine may also be administered by intramuscular injection in doses similar to those used orally.

Untoward and toxic effects. The untoward effects of ephedrine are nervousness, insomnia, tremulousness, vertigo, headache, tachycardia, palpitation, sweating, and a sensation of warmth. There may be difficulty in urination because of spasm of the vesical sphincter.

Vagolytic drugs that increase cardiac rhythmicity

Increase in cardiac rhythmicity may be anticipated after block of parasympathetic innervation to the heart. This is clinically established for two vagolytic drugs (atropine and methantheline), but all other parasympathetic blocking agents (at least twenty others) should also be effective.

Atropine. This alkaloid is indicated when increased vagal tone is a factor in the production of arrhythmias. This includes sinus slowing, partial A-V block, slow A-V nodal rhythm, and other cardiac mechanisms associated with slow heart rates. Atropine is also effective in syncope caused by cardioinhibition in the carotid sinus syndrome. Atropine is not used very often for these conditions, however, because its untoward effects are troublesome and vagal action may play only a minor part in the production of slow heart rates. Often cardiac slowing is the result of a direct depression of the S-A node, A-V node, or other pacemakers by anoxia, alterations in acid-base balance, and toxic or degenerative states, and these are not corrected by atropine.

Mechanism of action. The fundamental action of atropine is block of vagal nerve endings and diminution of vagal tone. The refractory period of the A-V node is shortened, and the atrial refractory period and conduction time are prolonged. There are three distinct phases of atropine action on the heart.[1] These include an initial vagotonic effect, a transient period of vagal imbalance at different levels of the conduction system, and a final prolonged period of parasympathetic block. The duration of these phases depends on the dose and method of administration—oral, subcutaneous, or intravenous.

Administration. The dose of atropine is 0.5 to 2 mg.; it is administered orally, subcutaneously, or intravenously.

Untoward and toxic effects. Toxic (and occasionally large therapeutic) doses of atropine may cause transient episodes of A-V heart block and A-V nodal rhythm; these effects are not particularly serious.[1] Untoward effects include dryness of the mouth, palpitation, blurring of near vision, restlessness, dry and hot skin, and disturbed speech; with increased grades of toxicity, these symptoms are more marked and there are a rapid, weak pulse, marked pupillary dilatation, hallucinations, delirium, coma, and eventually death.

Methantheline. The indications for the use of methantheline* are the same as for atropine. It has advantages over atropine in longer duration of action and fewer untoward effects in equally effective doses.

Mechanism of action. Methantheline exerts atropine-like effects.[16, 34, 44] After intravenous injection, there is tachycardia within 1 minute, apparently caused by vagal paralysis.

Administration. The usual route of administration is oral. The average dose for gastroenterologic use is 50 to 100 mg. every 6 hours, but for cardiac arrhythmias, larger doses are often necessary to obtain the desired effect.

Untoward and toxic effects. These include dry mouth, blurred vision, epigastric fullness, pyrosis, difficulty in urination, urinary retention, decreased libido, and constipation. Central nervous symptoms, such as restlessness, euphoria, and fatigue,

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*Banthine.

ppear in some patients. The drug is contraindicated in patients with cicatricial duodenal stenosis, achalasia, cardiospasm, organic pyloric obstruction, and coronary insufficiency. Neostigmine counteracts most of the untoward effects.

Alkalinizing agents that increase cardiac rhythmicity

In addition to the sympathomimetic and vagolytic drugs, alkalinizing agents are often effective in increasing cardiac rhythmicity. Among the more important members of this group are sodium lactate, which may be used in molar or half molar solution, and sodium bicarbonate.[15] In 1883 Ringer observed in the isolated perfused heart that when a ventricle had lost contractility, it could be restored in a short time by adding sodium bicarbonate to the saline solution.[52] Sodium bicarbonate acted by means of its alkalinity, because the addition of calcium hydrate or ammonium carbonate produced the same result. The effect of sodium bicarbonate was to neutralize the acid reaction produced by the metabolic processes of cardiac contraction.

Sodium lactate. Molar sodium lactate is most effective in the treatment of slow heart rates caused by or associated with hyperpotassemia[8, 13, 14] in the cardiac arrest of Stokes-Adams seizures, during anesthesia and surgical procedure, and in states accompanied by anoxia or acidosis or both.[14, 25]

Mechanism of action. Molar sodium lactate appears to exert its effect by one or more of the following: (1) a decrease in acidosis or the production of alkalosis, (2) a decrease in serum potassium,[17] (3) a vagolytic effect, and (4) a specific effect of the lactate ion. Because its action is based on a principle different from that of the vagolytic and sympathomimetic drugs, molar sodium lactate may supplement the use of these agents.

Administration. In patients with advanced potassium intoxication, 100 ml. of molar sodium lactate may be administered intravenously in 2 to 5 minutes, followed by intravenous infusion at the rate of approximately 30 to 60 drops per minute; the total amount given is dependent on the effects observed.[14] In patients with early electrocardiographic evidence of hyperpotassemia, an intravenous infusion may be given at the rate of 15 to 20 drops per minute until the desired effects are observed.

Since molar sodium lactate has a relatively short effect (about 2 hours), it is indicated in the Stokes-Adams syndrome only if there are frequent, repeated episodes or, perhaps, at the precise time of the attack. For repeated Stokes-Adams attacks, the production of a state of mild alkalosis with a low normal serum potassium level (about 3.5 mEq. per liter) is a desirable and effective prophylactic measure. This may be accomplished by the oral administration of molar sodium lactate in 90 ml. doses four times per day in addition to a low potassium diet. For multiple Stokes-Adams attacks, 40 to 80 ml. may be given intravenously in 1 to 2 minutes during the attack; this may be followed by an intravenous infusion at the rate of 60 to 150 drops per minute. As the ventricular rate increases, the rate of infusion should be slowed; and when the pacemaker maintains a satisfactory rate and there are no episodes of cardiac arrest, infusion should be stopped. In cardiac arrest during surgical operation, when contractions are slow but effective or manual compression maintains adequate circulation, molar sodium lactate may be given intravenously at the rate of 100 to 200 drops per minute (7 to 14 ml. per minute). However, if manual compression is ineffective or the initial intravenous dose of molar sodium lactate is ineffective, 20 to 40 ml. may be given slowly directly into the right ventricle at the rate of approximately 1 ml. per minute while manual compression is continued.

Untoward and toxic effects. Molar sodium lactate is contraindicated in the presence of alkalosis. Because of its osmotic effect, it increases the circulating blood vol-

ume. This and other aspects of its action tend to increase cardiac work. It should, therefore, be used with caution in patients with severe myocardial damage, particularly in incipient heart failure. In the initial stages, the effects of infusion should be monitored by the electrocardiograph, since it may produce ectopic beats, or increase their number if already present, or precipitate a rapid ectopic rhythm.

Barium chloride. Barium chloride was used in the past to increase cardiac rhythmicity in the presence of complete A-V block and Stokes-Adams seizures.[22] It increases both the force of contraction and the excitability of the myocardium. However, since barium chloride is toxic to heart muscle and may induce extrasystoles and even ventricular tachycardia, its use has been virtually abandoned.

Sympathomimetic drugs that secondarily decrease cardiac rhythmicity

The systemic pressor action of some sympathomimetic drugs initiates reflex cardiac slowing. The rise of blood pressure stimulates the baroreceptors in the aortic arch and carotid sinuses which in turn increase vagal tone. This often terminates premature beats and tachycardia of supraventricular origin. Four drugs have been used for this purpose: norepinephrine, methoxamine, mephentermine, and phenylephrine. From a practical standpoint, the general features of phenylephrine, as used to induce reflex slowing, are similar to methoxamine and will not be discussed in detail.

Norepinephrine. McGinn and Schluger,[45] as well as others, have found that in those cases in which it causes reversion to normal rhythm, norepinephrine (levarterenol)* usually acts by elevating the blood pressure.

Mechanism of action. Vasoconstriction induced by this drug appears to be most important in inducing the systemic pressor action. The vasoconstriction is selective in

that the coronary and cerebral vessels are spared or are weakly responsive. Receptors in the aorta and carotid sinus are stimulated by the elevation in blood pressure, thus inducing reflex stimulation of the vagus.

There is also evidence that suggests the decrease in cardiac excitability after norepinephrine may not be entirely reflex in nature, since it has been noted that these vasopressor drugs are also effective in man after the administration of atropine and in the laboratory animal after section of the vagi or complete denervation of the heart.[24] Vasopressor drugs which abolish arrhythmias may also cause serious arrhythmias if the blood pressure is raised too high, and they are less effective or ineffective as antiarrhythmic agents if the blood pH is lowered.[49, 59] In the presence of acidosis, therefore, it is also important to administer alkalinizing agents.

Administration. Norepinephrine is usually administered by continuous intravenous infusion; 4 ml. of the commercially available solution is diluted in 1,000 ml. with 5 per cent dextrose or 0.9 per cent sodium chloride solution to a final concentration of 4 μg of norepinephrine base per 1 ml. After the cardiovascular response to a test dose of 1 to 2 μg of base per 10 Kg. of body weight has been observed, the infusion rate is adjusted to obtain the desired pressor response (120 to 160 mm. Hg) as quickly as possible. Normally, infusion at the rate of 2 to 4 μg of base per minute (0.5 to 1 ml. per minute) is adequate. The blood pressure usually returns to control levels within 1 minute after cessation of the infusion; thus, the pressor response to the drug can be readily controlled. When sinus rhythm is restored, the infusion should be slowed or terminated altogether.[45]

Untoward and toxic effects. Norepinephrine sometimes produces transient ventricular fibrillation. However, in extensive use of norepinephrine in patients with severe myocardial damage, relatively few untoward effects have been encountered.

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*Levophed.

Hyperthyroid patients may be particularly sensitive to this agent.

Methoxamine. Methoxamine* has no direct cardiac stimulating action but induces intense reflex vagal activity. The rise of arterial pressure brought about by the constrictor action on systemic vessels activates the baroreceptors in the carotid sinuses and aortic arch.[3] In some instances, the drug has been used successfully to abolish atrial tachycardia.[20] It should be used with caution in myocardial shock, since it reduces cardiac output in the anesthetized dog,[2] as well as coronary blood flow and force of myocardial contraction. It may be administered intravenously in a dose of 5 to 10 mg., but it is preferable to inject it intramuscularly in a dose of 10 to 20 mg.

Mephentermine. Mephentermine† differs from all other sympathomimetic drugs in that it can stop or prevent some experimentally induced arrhythmias. Some clinical reports[60, 62] show that mephentermine is also effective in man. Intravenous injection is preferred. An initial dose of 45 to 60 mg. followed immediately by an infusion of 600 mg. in 500 ml. of 5 per cent dextrose in water produces an effective rise of blood pressure. Pressor effects last 30 to 50 minutes after intravenous injection.

Parasympathomimetic drugs that reduce cardiac rhythmicity

A more immediate way to increase vagal tone is to administer drugs which directly stimulate the parasympathetic nervous system. However, emetics also induce secondary parasympathetic effects.[31] For example, ipecac usually causes vomiting within 10 to 45 minutes, and cessation of tachycardia results in a large proportion of cases. It should be mentioned, however, that cholinergic drugs as well as intense vagal stimulation may sometimes induce atrial fibrillation.

Neostigmine. Neostigmine‡ is the drug of choice for stimulating parasympathetic

activity. It has been used successfully in paroxysmal supraventricular tachycardia to enhance parasympathetic effects,[5, 50] e.g., vagal stimulation induced by carotid sinus pressure. Neostigmine has been used successfully to prevent the onset of atrial fibrillation as well as for its treatment during surgical operation on the mitral valve. In the postural type of paroxysmal tachycardia, the drug may also be of value by counteracting sympathetic overactivity which develops on assuming the upright posture.[30, 57]

Administration. The dosage schedule is 0.25 to 0.5 mg. every 3 or 4 hours. In atrial paroxysmal tachycardia, the drug is injected subcutaneously in a dose of 0.5 to 2 mg. (1 to 4 ml. of a 1:2,000 solution). Onset of action is in about 20 minutes, reaching a peak at 1 hour and subsiding in 3 to 5 hours. It may also be administered intravenously, but this should rarely be done because of its toxic effects.

Untoward and toxic effects. The toxic effects of neostigmine, used in the doses described above, are relatively slight and include muscular twitching and intestinal hypermotility. Other reported evidence of toxicity, e.g., visual difficulties, curare effect, paralysis, weakness, and shocklike state, have not been observed by us.

Methacholine. Methacholine* is a powerful parasympathetic stimulant and is effective for treatment of atrial tachycardias.[55]

Administration. Methacholine is usually administered subcutaneously in a dose of 2.5 to 60 mg., average about 25 mg. Its effect becomes manifest in 1 to 2 minutes. Because of the marked hypotension that follows its administration, the patient should be in a recumbent position. Before the injection, a syringe filled with 0.5 to 1 mg. of atropine should be ready for immediate intravenous administration to counteract undesirable effects.

Untoward and toxic effects. These are relatively frequent and occasionally serious. The minor ones consist of flushing of the

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*Vasoxyl.
†Wyamine.
‡Prostigmin.

*Mecholyl.

face, hyperpnea, nausea, vomiting, and sweating. The most serious effect is a marked fall of blood pressure of 40 to 80 mm. Hg. In older patients and in those with coronary arteriosclerosis, serious complications may occur and occasional deaths have been reported. Prefibrillatory types of ventricular tachycardia and other aberrant ventricular complexes have been noted during the transitional period from atrial tachycardia to normal sinus rhythm. Because of the frequent untoward effects and since safer methods are available, neither methacholine nor acetylcholine, which acts in a similar manner, should be used in arrhythmias.

Digitalis glycosides

Digitalis belongs in a separate class because its antiarrhythmic action is unique in that the interference with conduction in the A-V node is the outcome of a vagal effect resulting from the action on the heart to increase the force of myocardial contraction, as well as a direct depressant action on the conducting mechanism.

The most important indication is atrial fibrillation. The ventricular rate in this arrhythmia can be decreased dependably by digitalis. In the treatment of atrial flutter, digitalis is the drug of choice since it is effective in 60 to 70 per cent of cases.

Mechanism of action. Digitalis exerts two effects: (1) direct depression of conduction and (2) reflex effects. The combination of these effects is seen in its action on atrial muscle, but since no vagal fibers are present in the human ventricle, the ventricular effects are usually considered to be the result of direct action alone. The reflex effects cause slowing of the heart and increase in atrioventricular conduction time (or P-R interval). In addition, digitalis exerts a direct effect on the A-V node not inhibited by atropine which increases the refractory period and prevents the rapid transmission of atrial impulses.

Untoward and toxic effects. One of the commonest causes of arrhythmias is digitalis toxicity, which can appear as almost

any type of arrhythmia originating in the atria, the ventricles, the atrioventricular conducting system, or the Purkinje fibers. Many circumstances are responsible for this: the widespread use of digitalis in cardiac patients (often in advanced heart disease), the frequent association of various degrees of electrolyte imbalance, and the difficulty of gauging the maintenance dose, especially in long-term therapy. Prophylactic measures to prevent digitalis toxicity involve cautious administration in patients with advanced heart disease, especially in the older age group, frequent observation of the patient (with electrocardiographic control), and examination for electrolyte imbalance, particularly during diuretic therapy. Hypopotassemia increases sensitivity to digitalis.

The treatment of digitalis toxicity is more difficult than its prevention. Therapy depends on the type and severity of the manifestations. In the presence of minor arrhythmias, e.g., partial A-V heart block, occasionally extrasystoles, or A-V dissociation, discontinuation of digitalis will usually suffice. In the presence of more serious arrhythmias, such as atrial tachycardia with block, nonparoxysmal nodal tachycardia, ventricular tachycardia, and other types of rapid ventricular rates, one should discontinue digitalis, determine the electrolyte pattern, and attempt to correct any imbalance observed. When serum potassium is low or normal, infusion of potassium may be given provided this is done with electrocardiographic monitoring. However, if no electrolyte determinations are available, procaine amide or quinidine is safer.[8]

Quinidine and other drugs that decrease cardiac excitability

The feature of this class of drugs is direct action on the heart muscle. Unlike digitalis, these drugs cause depression of all myocardial functions.

Quinidine. Quinidine is effective in the therapy and prophylaxis of a variety of ectopic rhythms. It is indicated in the treat-

ment of premature contractions (atrial, nodal, and ventricular) in paroxysmal atrial tachycardia, nodal tachycardia, atrial flutter, atrial fibrillation, and ventricular tachycardia.

Mechanism of action. Quinidine increases the refractory period of heart muscle by a direct effect, thereby decreasing myocardial excitability; it also slows conduction. The action of quinidine closely parallels its plasma concentration. A fall in arterial blood pressure occurs as the plasma level increases.

In man, quinidine produces a protein anabolic effect[28] and pronounced changes in carbohydrate metabolism.[56] These are manifested by inhibition of glucose utilization as shown by an impaired glucose tolerance curve. In myocardial slices and tissue homogenates, quinidine was found to (1) depress oxygen uptake,[58] (2) inhibit anaerobic glycolysis, (3) impair the oxidation of glucose pyruvate, citrate, fumarate, malate, and oxaloacetate,[58] and (4) decrease adenosine triphosphatase activity inhibiting bond utilization.[58] This depression of oxidative metabolism may be partially responsible for the pharmacologic effect of quinidine.[12]

The electrophysiology of quinidine is of interest. In very low concentrations, quinidine has two important effects on Purkinje fibers: (1) it reduces the slope of the action potential in fibers in which depolarization is spontaneous or induced by epinephrine or low calcium level and (2) it prolongs the refractory period of both Purkinje fibers and papillary muscle fibers.[40] These actions do not cause depression of resting potential. In toxic doses, quinidine may induce all the above. Quinidine resembles cocaine in altering the relation between membrane potential and the potential and the ability of the cell to increase sodium permeability. Thus, at a given degree of repolarization, there is less recovery from inactivation in the quinidine-treated fiber than in the normal fiber.

Increasing plasma concentration of quinidine causes increasing elevation of intracellular potassium which is not totally compensated by sodium loss, thus suggesting a net increase in intracellular ionic content.[23, 41, 42] Potassium, but not sodium, transcellular exchange is increased roughly in proportion to quinidine dosage. These apparently paradoxical findings are compatible with the views that (1) quinidine action on the heart results from increased mitrochondrial membrane permeability which allows release of energy systems and their action in the myocardial cytoplasm to increase transcellular potassium transport and (2) there are separate "pumping" mechanisms for sodium and potassium.[23]

Administration. The effect of quinidine depends not only on the plasma level but also on the effective binding power of quinidine to the heart muscle, the state of the myocardium, and the underlying cause of the ectopic rhythm. The presence of congestive failure, marked nervous tension, or fever may mitigate against conversion of an arrhythmia. Renal dysfunction may also modify dose-response relations.

To maintain an adequate plasma level, repeated doses of quinidine sulfate must be administered, and since the peak level is reached in about 2 hours, a common regimen is five doses of 0.3 to 0.4 Gm. per day at 2 hour intervals.[8] In this way, a plasma level of 40 per cent of the peak develops about 12 hours after the last dose. For conversion of an ectopic rhythm, it is advisable to use a relatively large dose of quinidine within a short time to obtain an effective plasma level. In 90 per cent of cases, conversion occurs at plasma levels below 10 mg. per liter.[54] After conversion to a normal sinus rhythm, effective plasma levels may be obtained by using the same dose of quinidine sulfate three or four times per day.

The intravenous preparations available are quinidine hydrochloride, quinidine lactate, and quinidine gluconate. The dose varies from 0.3 to 0.8 Gm. dissolved in 50 to 100 ml. of water or saline and administered slowly in 5 to 10 minutes under electrocardiographic control. Quinidine lactate

(0.65 Gm.) administered in a similar way produces maximal cardiac effect, as determined by changes in Q-T$_c$, within 15 minutes. Quinidine gluconate has been found most satisfactory for intramuscular use; in doses of 0.4 Gm. every 2 hours five times, it usually produces therapeutic effects without toxicity, while a single intramuscular dose of 0.8 Gm. may provide a satisfactory plasma level for 2 hours. When rapid therapeutic effect is desired with intramuscular quinidine, it should be given hourly; otherwise, at intervals of 3 to 4 hours.

Untoward and toxic effects. Quinidine is a protoplasmic poison. Considerable variation exists in tolerance to this drug. Quinidine may cause allergic reactions such as thrombocytopenic purpura,[48] cinchonism, gastrointestinal disturbances, convulsions, and in rare instances respiratory failure. Information on plasma level may be useful in cases of toxicity. The therapeutic level usually ranges between 4 to 10 mg. per liter; toxic effects occur at higher levels. Because of the variability of plasma levels, the electrocardiogram provides more information. In general, lengthening of the Q-T interval is a sign of therapeutic as opposed to toxic effect. Depression of atrial activity may ultimately lead to atrial standstill, and widening of the QRS complexes may precede ventricular flutter or fibrillation. When accompanied by hypotension and widened QRS complexes, the toxic action on the heart may frequently be reversed by stopping the drug or by cautious administration of molar sodium lactate and vasopressor agents. Sodium lactate infusion leads to (1) a rapid decrease in quinidine plasma concentration, (2) a decrease in serum potassium, (3) increased energy available for heart muscle (lactate effect), and (4) an increase in blood pressure with resultant improvement in renal function and tissue blood flow.

Chloroquine. Since its mechanism of action is similar to that of quinidine, chloroquine has occasionally been used as an antifibrillatory agent. Experimental data indicate that the drug may have advantage over quinidine in that it decreases conduction velocity only slightly and cardiac muscle recovers quickly after being depressed. There is little effect on systemic blood pressure and no marked depressive action on carbohydrate metabolism.[37] However, chloroquine has been found to be less dependable in controlling ectopic rhythms than quinidine.[9]

Administration. The initial dose is usually 1 Gm. per day, which is increased by 0.25 Gm. every third day until gastrointestinal side effects necessitate withdrawal. Maintenance doses of 0.25 to 1 Gm. per day have been used for as long as 1 year without noticeable toxic effects. The intramuscular dose is 30 mg. every 4 to 6 hours.

Untoward and toxic effects. Toxic effects are chiefly gastrointestinal. Large doses may cause lichenoid skin eruption,[31] mild and transient headache, and occasionally visual disturbances.[38] None of the symptoms are serious, and all readily disappear when the drug is withheld.

Procaine amide. The indications for procaine amide* are the same as for quinidine: extrasystoles and paroxysmal tachycardia of atrial, nodal, and ventricular origin.

Mechanism of action. The effects of procaine amide are very similar to those of quinidine. It exerts a marked effect through primary myocardial depression and leads to diminution of cardiac output, decrease in blood flow from the pulmonary vascular bed, and fall of pulmonary artery pressure. Like quinidine, procaine amide increases the refractory period of the atrium, prolongs conduction, and depresses the vagus; it also increases the refractory period of ventricular muscle. In the isolated atrium, procaine amide depresses the outward flux of potassium ions.[11] Because of the similarity in cardiac effects, it is likely many of the actions on oxygen uptake and enzymatic activity are similar to those of quinidine.

Very low levels of procaine amide prolong the effective refractory period of dog

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*Pronestyl.

papillary muscle fibers without producing any change in action potential.[40] Procaine amide also prolongs the effective refractory period of the Purkinje fibers and papillary muscle junctions without marked prolongation of the action potential. In concentrations known to affect Purkinje fibers, procaine amide does not cause an appreciable change in activity of the S-A node.[40]

Administration. Procaine amide may be administered orally, intramuscularly, or intravenously. The oral route is preferred. Intramuscular is preferable to intravenous injection because an effective plasma level is attained within ½ to 1 hour and hypotensive effects are relatively minor.[4] If the intravenous route is essential, as in the case of hypotension or shock, the infusion should be given in conjunction with vasopressor agents (e.g., norepinephrine), the first 500 mg. at the rate of 100 mg. per minute. Because the maximum effect of a single injection occurs within 4 minutes, subsequent injections should be given at the rate of 100 mg. every 4 minutes.[4] When intravenous infusion is utilized, the cardiac state of the patient should be monitored continuously by an electrocardiograph. Procaine amide should not be used in the presence of complete A-V heart block, even though numerous extrasystoles, ventricular tachycardia, or paroxysms of ventricular fibrillation are present. Doses sufficient to control the extrasystoles may also depress the normal cardiac pacemaker, thus inducing ventricular fibrillation or cardiac arrest.

Untoward and toxic effects. Serious toxic effects are referable chiefly to the cardiovascular system but may also involve the central nervous system. Minor reactions consist of gastrointestinal symptoms; these do not necessarily indicate discontinuance of the drug. Anorexia, nausea, and vomiting are not infrequently observed in patients who are receiving large oral doses of procaine amide or who have received it for a long period of time. Unlike procaine, procaine amide produces minimal central stimulation, usually after rapid intravenous administration. Although the intramuscular injection of procaine amide may lead to a fall of blood pressure, it is less frequent and relatively slight compared to the fall observed after intravenous injection. The toxic effects of procaine amide on the heart are similar to those of quinidine and can be reversed by molar sodium lactate and vasopressor agents.[11]

Potassium salts. Potassium salts are effective in terminating ectopic rhythms, such as atrial, nodal, and ventricular extrasystoles, and the respective tachycardias, in a manner similar to quinidine and procaine amide. Potassium salts have no particular advantages over quinidine and procaine amide except (1) in the presence of arrhythmias associated with digitalis toxicity, (2) in the presence of arrhythmias associated with hypopotassemia, and (3) in the occasional instances in which the arrhythmias fail to respond to quinidine and procaine amide.

Mechanism of action. Potassium increases the refractory period of heart muscle and slows conduction.

Administration. Because of the potential toxicity of potassium, the salts should be administered with caution, especially in the presence of renal insufficiency and advanced heart disease. Serum potassium level determined before infusion may be helpful: in the presence of shock and anoxia, the serum potassium level may be high, thus infusion would be dangerous. If the salts are given in an acute arrhythmia with rapid ventricular rate, continuous electrocardiographic monitoring is mandatory. If toxic effects occur, they may be reversed immediately by intravenous infusion of molar sodium lactate or 50 per cent glucose. The oral route is not used because relatively slow absorption makes electrocardiographic monitoring difficult.

Untoward and toxic effects. Toxic effects consist of a fall of blood pressure because of direct myocardial depression accompanied by peripheral vasodilatation. There may also be depression of atrial activity, increase in the intraventricular conduction time, and development of a slow idioven-

tricular rhythm which finally leads to cardiac standstill.[7]

Antihistamine drugs. It has been shown experimentally that some antihistamines,[26, 27] e.g., tripelennamine* and diphenhydramine,† exert effects on heart muscle similar to those of quinidine. Although no extensive trials have yet been carried out, scattered reports suggest that antihistamines have definite antifibrillatory action in man. Although they are less effective than quinidine in controlling supraventricular tachycardia or atrial fibrillation, trial is justified in patients who cannot tolerate quinidine or similar drugs.

Papaverine. Papaverine is closely related to quinidine. In large doses, it acts like quinidine, that is, it depresses atrioventricular conduction and leads to A-V block, sinus slowing, or standstill with A-V nodal escape. Papaverine often abolishes extrasystoles and paroxysmal ventricular tachycardia, but it is inferior to quinidine and procaine amide and is rarely used for antiarrhythmic effect.

Miscellaneous antiarrhythmic drugs

Methimazole and propylthiouracil. These antithyroid drugs are useful in cases of hyperthyroidism in which arrhythmias have developed. In euthyroid patients with frequent or persistent experience of premature beats and paroxysmal tachycardias resistant to other therapy, the administration of these drugs may help. They reduce sympathetic activity and thereby decrease the cardiac response to catecholamines.

Administration. The initial dose of propylthiouracil in hyperthyroidism is 300 to 400 mg. per day. The maintenance dose varies from 50 to 300 mg. per day. Methimazole is given initially in a dose of 40 to 60 mg. per day and may later be reduced to 10 to 20 mg. for maintenance.

Untoward and toxic effects. These drugs may cause serum sickness, agranulocytosis, dermatitis, enlargement of lymph nodes and salivary glands (Mikulicz's disease), and hepatocellular jaundice. These reactions can usually be detected at the stage of fever and arthralgia, or pruritus, before they progress to more serious forms. There is a higher incidence of untoward reactions after propylthiouracil than after methimazole.[31]

Adrenolytic drugs. Since ectopic rhythms frequently result from sympathetic overactivity, attempts have been made to counteract this action directly. Dihydroergocornine has occasionally been used in refractory cases of paroxysmal atrial tachycardia. However, they are rarely used for this purpose since simpler and more effective measures are available and since these drugs have caused untoward effects.

Reserpine. Reserpine has been used to slow sinus tachycardia, regardless of the cause, and as an adjunct in the treatment of certain ectopic tachycardias. The alkaloids of Rauwolfia slow the heart rate and depress atrioventricular conduction.[33, 36, 61] An important aspect of their action is depletion of sympathomimetic amines, especially norepinephrine, in heart muscle. Prompt reversion to normal sinus rhythm has been observed in paroxysmal atrial fibrillation, although there is no effect in chronic cases. Since reserpine decreases blood pressure, it should be used cautiously in hypotensive patients. Because of its tendency to aggravate this state, its use is best avoided in patients with peptic ulcer.

Diphenylhydantoin. Diphenylhydantoin* is an anticonvulsant. However, digitalis-induced ventricular arrhythmias in dogs have been favorably influenced by the drug. It reduces ventricular ectopic activity in acute myocardial injury induced by coronary artery ligation[35] and causes reversion of aconitine-produced atrial fibrillation to normal. Occasionally, it has exerted a favorable effect on ectopic rhythms in man.

Hydroxyzine. Hydroxyzine† has recently been used for its antiarrhythmic effect. It

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*Pyribenzamine.

†Benadryl.

*Dilantin.

†Vistaril.

has been suggested that its ataractic action is the basis for this effect.[19] Some reports indicate that hydroxyzine is less dependable than quinidine or procaine amide.[43]

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Symposium on the experimental pharmacology and clinical use of antimetabolites

Part VII. The spirolactones

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In the short period of time since the discovery of aldosterone by Simpson, Tait, and Bush,[77] it has become apparent that excessive production of this steroid plays an important role in the pathogenesis of several disease states. Hyperaldosteronism may result from intrinsic disease of the adrenal glands (primary aldosteronism) with or without overproduction of other adrenal corticoids.[15, 21, 22, 58] Of greater importance, in terms of incidence, are those disorders that involve primarily organs other than the adrenal glands but owe some of their abnormal features to a secondary excess of aldosterone production by the adrenal glands. Such secondary aldosteronism is present in most conditions which are associated with pathologic accumulations of extracellular fluid, such as nephrosis,[53, 62] congestive cardiac failure,[20, 53, 79] cirrhosis with ascites,[12, 13, 53, 98] idiopathic hypoproteinemia,[4] kwashiorkor,[*] and idiopathic edema.[54, 56, 57, 74] Hyperaldosteronism has also been reported in essential hypertension,[29, 31] but the significance of this observation is not clear.

In primary aldosteronism, adrenalectomy

°D. H. P. Streeten: Unpublished observations.

will reverse most or all of the abnormal features, and in some diseases associated with secondary aldosteronism, the operation has been shown to abolish or greatly to reduce those features (e.g., edema) which result from excessive aldosterone activity.[30, 34, 60] These findings encouraged the hope that a drug would be found which would either reduce the adrenal production of aldosterone or antagonize the effects of excessive amounts of this corticoid. Amphenone B was the first substance tested for ability to suppress adrenal cortical production of aldosterone and other steroids.[35, 70] The use of amphenone B did result in reduction of ascites in patients with cirrhosis,[89, 97] but toxic effects contraindicated long-term administration. In the past 2 years another compound, known as SU-4885, has been introduced; this drug blocks one of the enzymatic steps (11β-hydroxylation) concerned in normal adrenal steroidogenesis and in this way reduces the adrenal production of aldosterone and other 11-oxygenated corticoids.[52] Preliminary observations indicate that this agent is potentially useful in counteracting the manifestations of secondary aldosteronism.[18] The spirolactones also reverse the features of hyperaldosteronism, but by an
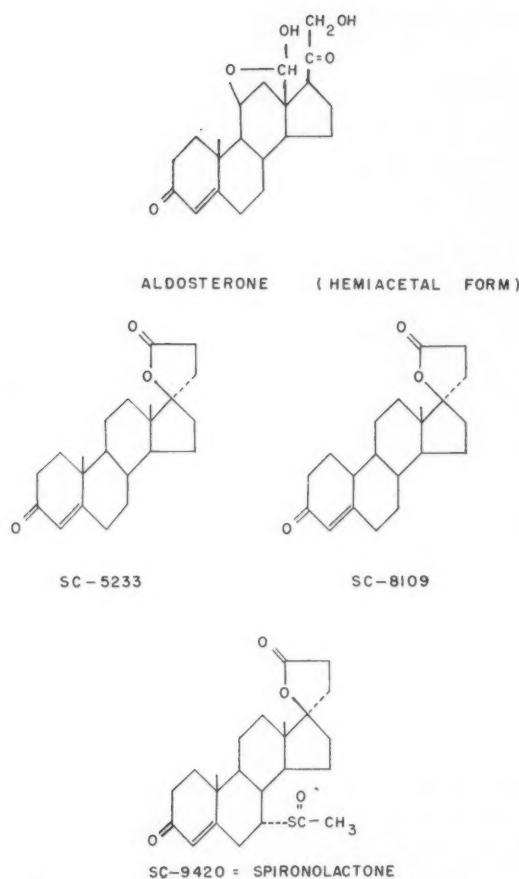
Fig. 1.

entirely different mechanism which appears to involve competitive inhibition of the effects of aldosterone. Extensive experimental work and clinical use have shown that these drugs are effective and frequently valuable in the treatment of conditions associated with excessive production of aldosterone. Their pharmacology and clinical usefulness will be discussed in this paper.

Pharmacology of the spirolactones

Relationship between chemical structure and pharmacologic actions. The steroidal 17-spirolactones contain, as their name implies, a 5-membered lactone ring attached at the C-17 position in the steroid nucleus. The similarity of structure between these compounds and the hemiacetal form of aldosterone is shown in Fig. 1. The first member of this series of compounds shown to be biologically active as an antagonist of the effects of aldosterone and

desoxycorticosterone acetate (DCA) was 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propionic acid lactone (SC-5233).[11] Biologic activity was enhanced in the corresponding 19-nor compound (SC-8109)[11] but was reduced by changing the 3-oxo-4-ene system to 3-hydroxy-5-ene, 3-oxo-4,5-dihydro, or an aromatic ring A.[10] Both expansion of the lactone to a 6-membered ring and introduction of a double bond at C-20 and C-21 reduced the biologic activity of the compound.[10] Addition of an acetylthiogroup at the 7α position resulted in a compound (SC-9420) that was found to be as active by oral as by parenteral administration and was approximately five times as potent as SC-8109 when administered by the oral route.[9, 39] SC-9420 has been assigned the generic term spironolactone.[*] The synthesis of many of the spirolactones has been described by Cella, Brown, and Burtner.[10]

Pharmacologic action on the kidney. The spirolactones increase urinary excretion of Na^+, Cl^- and water and reduce the excretion of K^+ (usually), ammonia, titratable acid, and phosphate in normal subjects and in patients with edema.[3, 16, 49, 50, 73, 96] Kagawa, Cella, and Van Arman[41] first showed that the changes in urinary sodium and potassium are not seen when the spirolactones are administered alone to adrenalectomized rats but become evident when the animals are given DCA or aldosterone concurrently. Evidence obtained in dogs and in human subjects has adequately confirmed the view that the spirolactones exercise their characteristic effects only in the presence of the adrenal glands or when aldosterone, DCA, or a steroid with similar biologic properties is administered.[3, 49, 51, 81]

Glomerular filtration rate (GFR) is either unaffected by the administration of the spirolactones[8, 96] or may tend to fall slightly.[28, 96] The drugs do not change the effective renal plasma flow.[96] The reciprocal effects on the excretion of Na^+ on the one hand and K^+ plus H^+ on the other suggest

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[*]Aldactone.

that the natriuretic effect of these compounds is mediated through an action at the distal tubule. The fact that diuresis induced by these agents in patients with edema is associated with chloruresis as well as natriuresis, however, indicates that the effects are probably partly mediated by inhibition of the reabsorption of sodium and chloride in the proximal renal tubule. A slight increase in the free water clearance has been observed by Wiggins and associates[96] in four out of five studies, by Bartter,[3] but not by Laragh.[48]

The natriuretic and diuretic actions of the spirolactones are greatly enhanced by the concomitant administration of chlorothiazide.[7, 34, 50, 64, 65] This enhancement might be attributable to the difference in the sites of action of the compounds, the spirolactones, like aldosterone, probably acting predominantly at the distal tubule, chlorothiazide mainly at the proximal tubule.[91, 92] Morrison[64] found that further potentiation of the diuretic effects, frequently of such magnitude as to be of real clinical significance, resulted from the addition of a glucocorticoid such as prednisone or 6-methyl prednisolone. It is not known whether or not this action of the glucocorticoids results from the increase in GFR which these substances are known to cause or from an effect on the aldosterone production rate. Administration of supplementary potassium also increases the sodium-losing effects of the spirolactones,[90] at least in some patients with cirrhosis and ascites.

Liddle[50, 51] has pointed out that the spirolactones differ from most other diuretic agents in reducing rather than increasing the potassium excretion, an effect which may be clinically desirable in a chronically ill patient with potassium depletion.

In large doses, SC-8109 has been shown to exercise a DCA-like action on the urinary Na$^+$/K$^+$ ratio of adrenalectomized rats. SC-8109 has approximately 0.1 to 0.2 per cent of the action of DCA by this criterion.[38]

Effects on salivary and fecal electrolytes. Spirolactone administration usually increases the Na$^+$/K$^+$ ratio of saliva.[34, 46, 82-84]

This reduction is more evident when the ratio is measured in the afternoon a few hours after the last dose of the drug than in the morning 8 to 12 hours after the last dose was administered.[84] Slater and associates[81] found that the Na$^+$/K$^+$ ratio in the feces increased in 2 patients during treatment with SC-8109.

Action on aortic contractility. An effect of the spirolactones that has been observed in vitro is their potentiation of the contraction of a strip of rabbit aorta immersed in epinephrine.[6] The spirolactones resemble desoxycorticosterone (DOC) and other steroids in this action, equipotent doses of the substances being DOC:1, SC-5233: 1.5, SC-8109:8, hydrocortisone:5, aldosterone:7, and 2-methyl-9α-fluorohydrocortisone:>25.

Progestational activity. By the subcutaneous route of administration, SC-8109 has been shown to be approximately one-half and SC-5233 one-tenth as potent as progesterone in inducing progestational proliferation of the endometrium of estrogen-primed immature female rabbits.[36] Spironolactone, on the other hand, had no progestational activity when given to the same animals in doses of 0.5 and 1 mg. by subcutaneous injection.[9, 40] SC-8109 is approximately equipotent with 19-nor-ethinyl-testosterone in the same assay when both substances are given by mouth.[36] In its ability to reverse the effects of 12 μg of DCA on the urinary Na$^+$/K$^+$ ratio of rats, 0.25 mg. of SC-5233 is as effective as approximately 1.8 mg. of progesterone.[41]

Effects on nitrogen balance. Effects on nitrogen balance are negligible in most circumstances. However, Faloon[23] has observed the occurrence of positive nitrogen balance in patients with cirrhosis and ascites under treatment with SC-8109. This effect may be the mechanism of the striking restoration of normal muscle mass which Gantt[25] observed after treatment with spironolactone in a patient with cirrhosis and which Henley, Streeten, and Pollard[34] described in a severely cachectic female with cirrhosis and ascites during her

dramatic general improvement after bilateral adrenalectomy. The observations seem to suggest that excessive aldosterone activity in some way contributes to the state of protein depletion and cachexia in liver disease with ascites.

Effects on adrenal function. The urinary 17-ketosteroids are unchanged[16] but the excretion of 17-hydroxycorticoids has been reported to be reduced by treatment with SC-8109.[7, 16, 28] This results from great reduction and sometimes almost complete disappearance of the conjugated fraction of the urinary 17-hydroxycorticoids.[7] Such a change might represent an effect on the hepatic metabolism rather than on the rate of adrenocortical production of hydrocortisone. In studies of the effects of the spirolactones on urinary corticoids, it is important to appreciate that the excretory products of spironolactone interfere with the determination of 17-hydroxycorticoids by the Porter-Silber reaction,[51] with the measurement of the 17-ketosteroids by the Zimmerman reaction,[8] and with the chromatographic determination of aldosterone by the method of Neher and Wettstein.*[8] When aldosterone secretion rates have been determined by the isotope dilution technique in patients receiving spironolactone or when rates of excretion of aldosterone have been measured chromatographically during treatment with SC-8109, the results have been inconsistent. Thus, the agents have been reported to increase, to decrease, or to leave unchanged the secretion rates or the excretion of aldosterone in patients with primary aldosteronism.[5, 7, 28, 48, 69, 99] These variable findings might simply reflect spontaneous day to day fluctuations in aldosterone excretion by patients with primary aldosteronism[17] in whom the administered drug had no effect on autonomous secretion of aldosterone by an adrenocortical tumor. Where measurements have been made in patients with secondary aldosteronism, the results have been more consistent, the spirolactones increas-

ing aldosterone secretion rates or excretion in normal subjects on a low sodium intake[51] and in patients with cirrhosis[7, 71, 81] and with nephrosis.[28, 81] Singer[78] has shown that SC-8109 accelerates the rise in the rate of secretion of aldosterone into the adrenal vein of rats on a restricted intake of sodium. On the other hand, SC-8109 appears not to affect the rate of secretion of aldosterone or corticosterone into the adrenal vein blood of rats with nephrosis induced by injections of aminonucleoside.[19] In these experiments, it is conceivable that the rates of aldosterone secretion were maximal in the nephrotic rats and thus could be increased no further by treatment with SC-8109.

Slater and colleagues[81] have observed a paradoxic fall in the excretion of aldosterone extractable at pH 1 in 2 patients with cardiac failure during the first 3 days of treatment with SC-8109.

DCA-induced, metacorticoid, and adrenal regeneration hypertension. Kagawa, Sturtevant, and Van Arman[42] have reported SC-5233 will significantly reduce the hypertension occurring in young rats both during treatment with DCA pellets and in the state of "metacorticoid" hypertension, after removal of these pellets. The hypertension which occurs during adrenal regeneration in rats[80] is prevented by chronic administration of SC-5233.[88]

Androgenic and anabolic actions. Kagawa[39] has found that SC-8109 has androgenic and anabolic activities approximating 1 per cent and 2 to 5 per cent of the corresponding activities of testosterone propionate in castrated male rats. No such action could be shown for spironolactone in doses of 5 mg. daily for 7 days.

Other pharmacologic properties. Kagawa's[39, 40, 42] studies have shown that SC-5233, SC-8109, and spironolactone are all devoid of demonstrable estrogenic activity and devoid of hydrocortisone-like activity on the granuloma pouch, resistance to infection with Coxsackie virus, the peripheral eosinophil count, or liver glycogen content.

°M. C. Minick: Personal communication.

Mode of action of spirolactones

Kagawa, Cella, and Van Arman[41] postulated that SC-5233 acted as a competitive inhibitor of DCA and aldosterone, since (1) the spirolactone had no demonstrable action in adrenalectomized rats unless DCA or aldosterone was administered concurrently, (2) equal degrees of blocking of the effects of DCA resulted from similar ratios of the administered doses of DCA and SC-5233, and (3) the effects of administered DCA were blocked increasingly as the ratios of SC-5233 to DCA were increased, while the block was reduced by decreasing the ratio SC-5233/DCA. This interpretation of the mechanism of action of the spirolactones was strongly supported by Liddle's[49, 51] findings that the drugs reverse all of the known actions of aldosterone on renal function, including the effects on Na^+, K^+, Cl^-, titratable acidity, phosphate, and ammonia excretion and that in dogs and human beings, as in rats, the spirolactones have no detectable action in the absence of the adrenal glands unless a mineralocorticoid is administered. Further support for competitive inhibition of aldosterone as the mode of action of the spirolactones is provided by Liddle's[51] observation that the drugs produce effects virtually identical with those resulting from reduced aldosterone synthesis under the influence of the 11β-hydroxylase inhibitor, SU-4885, yet do not reduce aldosterone production, which, on the contrary, is increased by their administration. The observed extrarenal actions of the spirolactones on salivary and fecal electrolyte concentrations are those which would be expected from an inhibitor of the effects of a circulating hormone, aldosterone.

On the other hand, there is evidence that some effects of the spirolactones do *not* result from antagonism of circulating aldosterone-like hormones, e.g., the effect on the conjugated fraction of the urinary 17-hydroxycorticoids.[7] Moreover, even the important diuretic actions of the drug might possibly be explained as effects which required the presence of "permissive"

amounts of mineralocorticoids acting at approximately the same site.[48] If the spirolactones act purely as competitive inhibitors of aldosterone, it is difficult to understand the reason for the usual observations that the increments in urinary sodium excretion are very similar whether the aldosterone secretion rate has been low or greatly elevated.[16, 48, 71, 81] Thus, whereas it was claimed in the early publications that the spirolactones had no significant natriuretic effect in subjects on a high sodium intake,[49] Conn and coauthors[16] found that the extent of the negativity of sodium balance induced by SC-8109 was approximately equal on sodium intakes of 9, 200, and 475 mEq. per day in normal subjects. These findings have subsequently been largely confirmed by Liddle[51] and by Bartter,[3] who believe nonetheless that the natriuresis seen during the administration of spirolactones might still result from antagonism of the persistent, though much reduced, output of aldosterone. While these pieces of evidence throw some doubt on the theory that the spirolactones act by competitive inhibition of endogenous aldosterone, the theory has by no means been disproved, is still supported by a wealth of strong evidence, and certainly serves as a useful working hypothesis.

Therapeutic usefulness of the spirolactones

General considerations.

Lag between onset of treatment and therapeutic response. It has been observed by several individuals[37, 64, 82] that the natriuresis and the beneficial clinical results from therapy with the spirolactones are frequently slight in the first few days of treatment but tend to increase with continued therapy. These features differ strikingly from the changes seen during the administration of some of the commoner diuretic agents in present or recent use, such as acetazolamide and the thiazide diuretics, which characteristically produce a dramatic diuresis on the first day of treatment, with diminishing effects as the treatment is continued. Such differences between the ac-

tions of diuretics make it very difficult to decide, either from the short-term experiments in the pharmacology laboratory or from acute balance studies in man, how valuable a diuretic agent is likely to prove in the long-term clinical management of edema. The important clinical implication of the differences is that the thiazide drugs are likely to prove valueless unless diuresis starts on the first day of their administration, whereas this is not true of the spirolactones, particularly when they are used in the usual dosage of 300 to 500 mg. daily. Thus, even if after 7 days of treatment spironolactone has induced only a slight to moderate increase in urinary sodium output or a small loss of weight on a low sodium diet, continued treatment will often be rewarded by progressive and clinically significant reduction of edema. It is possible that the lag between the onset of therapy and clinical benefit may be shortened by starting treatment with larger doses such as 800 or 1,200 mg. daily for 3 to 6 days, but at present there is little conclusive evidence to confirm this clinical impression. If, on the other hand, there is no evidence of natriuresis or weight loss after the administration of 800 to 1,200 mg. of spironolactone daily for a week, it is reasonable to conclude that other agents will have to be used in addition to, or instead of, spironolactone. It should be mentioned that when spironolactone is used for the treatment of hypertension, not even a lack of response at the end of 1 week is sufficient to indicate that continued use of the drug will be of no value, since the greatest hypotensive effects of the spirolactones are frequently seen only after several weeks of treatment.[37]

Use of adjuvant measures. There is now abundant evidence that many patients with edema who are refractory to treatment with spironolactone alone or to chlorothiazide alone will undergo a very satisfactory diuresis when the two drugs are given together.[14, 34, 64, 66, 76] However distasteful it might be to indulge in such polypharmacy, there is little doubt of its practical value

in these difficult therapeutic problems. A variety of adjuvant measures may be tried in patients who fail to respond to spironolactone alone. A low sodium diet should always be used, since the chances of accomplishing a negative sodium balance and consequent weight loss are enhanced by reducing the sodium intake.[23] Since the administration of potassium chloride increases the sodium losses induced by spironolactone,[90] it is worth while to use potassium supplements routinely in daily doses of approximately 45 to 80 mEq., either as Randall's solution (5 to 9 ml. three times daily) or as potassium chloride tablets (1 to 2 Gm. three times daily) with or immediately after meals. When this is done, however, it is always wise to measure the serum K^+ concentration at least once or twice a week, since hyperkalemia may result from even these small supplements of potassium in patients receiving spironolactone.[90] When spironolactone fails to induce natriuresis or weight loss in patients receiving a low sodium diet and potassium supplements, the addition of a thiazide diuretic in conventional doses will usually produce the desired effect.[14, 33, 34, 64, 66, 83] Alternatively, one of the glucocorticoids such as prednisone, prednisolone, or 6-methyl prednisolone in doses of 20 to 50 mg. daily will frequently augment the natriuretic effects of spironolactone to induce a satisfactory loss of edema and ascites.[14, 64] The use of the glucocorticoids is particularly advantageous in patients with hyponatremia in whom the serum sodium may frequently be restored to normal levels by these means.[64] Shaldon, McLaren, and Sherlock[76] have recently reported that intravenous infusions of 200 Gm. of mannitol, given as a 10 per cent solution over 6 hours, considerably augmented the natriuresis resulting from administration of spironolactone and chlorothiazide and resulted in loss of previously refractory, long-standing ascites in 7 out of 9 patients. Organic mercurials may be used with good effect too, in combination with spironolactone and/or a glucocorticoid.[64] Morrison[64] has shown that in pa-

tients with cirrhosis and resistant ascites, individual clinical trial may be necessary to disclose which of the various possible combinations of drugs results in the greatest diuretic response.

Use of spirolactones in specific diseases.

Cirrhosis with ascites. A number of authors have used the spirolactones with success in the treatment of cirrhosis with ascites.[7, 14, 23, 25, 26, 32-34, 43, 46, 59, 64, 65, 76, 81, 82, 90, 95] Spironolactone will frequently bring about natriuresis and loss of weight with complete disappearance of ascites and edema in patients who are refractory to other diuretic agents. When spironolactone alone fails to produce a significant improvement, as happens not infrequently, its use together with potassium supplements and thiazide diuretics and/or a glucocorticoid,[64] and organic mercurial,[64] or mannitol infusions[76] will succeed in the great majority of patients previously resistant to diuretic therapy. Thus, in groups of patients all refractory to the thiazide or mercurial diuretics, administration of various combinations of these drugs, always including spironolactone and chlorothiazide, induced loss of all or almost all evidence of ascites in 15 of 18 patients treated by Morrison,[64] 6 of 9 treated by Henley,[33] 9 of 11 treated by Gantt,[25] 7 of 9 treated by Shaldon, McLaren, and Sherlock,[76] and all 8 patients treated by Clowdus and associates.[14] In some of these patients, fluid retention tended to recur when the treatment was stopped, but in others,[34, 76] the spirolactone apparently rendered the patients responsive to small doses of chlorothiazide, which have sufficed to keep them free of detectable ascites as long as the follow-up has continued, for many months after the treatment with spirolactone had been discontinued. Most authors[23, 25, 64] doubt that spirolactone therapy increases the blood ammonia concentration or induces hepatic coma. On the contrary, Clowdus and associates[14] have reported improvement in the nervous complications in 2 patients who had evidence of impending hepatic coma before treatment with spironolactone was started.

Nephrotic syndrome. It has been shown in patients with nephrosis that the spirolactones are capable of increasing urinary sodium and water excretion with consequent weight loss and reduction or complete disappearance of the edema.[28, 50, 81] Beneficial results have been observed even when the nephrotic syndrome was associated with histologic evidence of mixed glomerulotubular nephritis or with diabetic nephropathy.[28] Mild degrees of azotemia and hypertension have not prevented good diuresis during treatment with the spirolactones.[28, 81] Considerable reduction in the endogenous creatinine clearance, to levels as low as 15 to 30 ml. per minute per 1.73 sq. M., did not preclude the occurrence of effective diuresis with spirolactone therapy, despite the fact that such treatment usually caused a further fall in the creatinine clearance.[28] There are, as yet, no published reports that enable one to compare the effectiveness of spirolactone therapy with glucocorticoid therapy in patients with nephrotic edema. Neither has the follow-up been adequate to show whether lasting remissions may be induced by spironolactone. Further studies are needed before it can be decided what place, if any, spironolactone will have in the management of patients with the nephrotic syndrome.

Congestive heart failure. Patients with congestive heart failure have shown a variable response to treatment with the spirolactones. Where uremia has been present because of concomitant renal disease, SC-8109 has been quite ineffective.[50] Short courses of therapy with relatively low doses have increased urinary Na+ output slightly without reducing edema significantly.[24, 81] However, when the treatment has been prolonged, it has usually been found that natriuresis continued and that gratifying diuresis could be brought about.[7, 8, 48, 64, 68, 81] Thus, for instance, a man of 72 years with arteriosclerotic heart disease, in whom severe congestive failure was consistently refractory to mercurials and to chlorothiazide, responded when spirolactone treatment was added with a loss of weight from 240

to 128 pounds and great clinical improvement over the course of 2 months.[64] Furthermore, it may be necessary to use larger than the usual doses of spironolactone in some patients, such as the one reported by Cejka, de Vries, and Borst,[8] who continued to gain weight on 600 mg. of spironolactone daily but lost weight on 1.2 and 2.4 Gm. daily. Potentiation of the diuretic effect of other agents by the spirolactones has been observed in patients with congestive cardiac failure, as in those with cirrhosis and ascites.[64] It is not known whether patients with different types of heart failure would differ in their response to treatment with spironolactone, and it is still far too early to predict how frequently spironolactone will prove to be useful in the treatment of congestive heart failure. However, there is already good reason to believe that spironolactone will be valuable in some patients who respond inadequately or who are resistant to treatment with other diuretic agents. In such patients spironolactone should be used together with a low sodium diet and chlorothiazide or a mercurial diuretic. The dosage necessary would depend on the observed response but should be increased, if necessary, to 800 or 1,200 mg. daily.

Essential hypertension. Hollander[37] has shown that a significant proportion of patients with essential hypertension (68 per cent of his selected series of 32 patients) experienced a fall in systolic and diastolic blood pressure provided treatment with spirolactones (alone or together with other agents) was continued for 1 to 4 months. The improvement was seen even when sodium intake was unrestricted and apparently was not related to the magnitude of change in the plasma or extracellular fluid volume, as it was not immediately reversed by infusing plasma or physiologic saline. The reduction in pressure averaged 21/11 mm. Hg when spirolactone was used alone and ranged from 15/10 to 55/30 when spironolactone was used together with other drugs. Smaller falls in blood pressure were found with the spirolactones by

Laragh.[48] Genest[28] has pointed out that patients with renal hypertension very seldom experience a fall in blood pressure on spironolactone. In the present-day plethora of effective hypotensive drugs, the place of spironolactone in the treatment of hypertension will be difficult to assess. It appears to be valuable in the potentiation of chlorothiazide in some patients, and both Hollander[37] and Genest[28, 29] believe that the spirolactones may represent an advance in the treatment of arterial hypertension.

Since a therapeutic trial with spironolactone is likely to be inconclusive unless it continues for 2 to 4 weeks or longer, it would seem that the use of spironolactone in hypertension will not be practical prior to the discovery of some means of selecting in advance patients who are reasonably likely to respond to the drug.

Primary aldosteronism. The weakness, hypokalemia, and alkalosis of primary aldosteronism are all readily reversed by the spirolactones, as Salassa, Mattox, and Power[75] have shown and as others have confirmed.[*,44, 55] The patient of Conway and Streeten[*] experienced a fall in blood pressure, too, but the relatively poor hypotensive response of some patients with primary aldosteronism to adrenalectomy (Milne, Muehrcke, and Aird[63]) indicates that restoration of a normal blood pressure cannot be expected to result from spironolactone therapy in all of these patients. Furthermore, since the majority of patients with primary aldosteronism have one or more adrenocortical adenomata, the long-term use of spironolactone is not likely to replace adrenalectomy as the treatment of choice in this condition. However, spironolactone may prove to be of value to patients with primary aldosteronism in three circumstances. First, its administration may be useful in the diagnosis of primary aldosteronism from such conditions as "potassium-losing nephritis," in which the hypokalemia will presumably be unaffected by

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[*]J. Conway and D. H. P. Streeten: Unpublished observations.

the drug. Second, the administration of spironolactone in doses of 800 to 1,000 mg. daily for several weeks improves the general condition of patients with primary aldosteronism and might be of value in avoiding the anesthetic complications which have been encountered in operating on them.[27] And third, spironolactone treatment will be useful in patients who either refuse operation or cannot be operated upon for any reason—such as a patient of Conway* who needed cardiac massage when his heart stopped beating during a simple cystoscopy a few months before the diagnosis of primary aldosteronism was made. This man's blood pressure has been well controlled, serum electrolytes have been maintained within normal limits, and his strength has improved greatly in the 7 months since the treatment with spironolactone was started.

Idiopathic edema. Among patients with idiopathic edema, it is possible to distinguish two groups, in both of which the upright posture appears to be concerned in the abnormal retention of fluid. In the first of these groups, the patients have a severe impairment in excretion of a water load when in the upright posture, an impairment corrected by ethanol and by recumbency. From the limited studies on these patients, so far, it appears that they excrete normal, high normal, or slightly increased amounts of aldosterone in the urine and usually respond poorly to spironolactone administration.[85] In the second group of these patients, there is an abnormal degree of retention of sodium and water in the upright posture and the aldosterone excretion is elevated, often as high as 50 μg per day on a normal sodium intake.[87] Seven patients of this type have been treated with spironolactone and all responded with a fall in morning body weight, owing to increased sodium and water excretion.[84, 87] Edema was greatly reduced, and there was a gratifying lessening of the discomfort as well as

of the paresthesias, headaches, and impaired powers of mental concentration which were frequent complaints in these patients. Their general sense of well-being improved while spironolactone was being administered and deteriorated when placebo tablets were substituted. Daily doses of 400 to 700 mg. of spironolactone was usually sufficient, but in 1 patient it was necessary to give as much as 1.2 Gm. daily in order to elicit a satisfactory clinical response. Good results could be achieved with lower doses if chlorothiazide was administered concurrently. The amphetamines, particularly dextroamphetamine, in doses of 20 to 30 mg. daily reduced the degree of weight gain during the daytime in the upright posture to an extent which was statistically significant. While there is no doubt that the mildly affected patients are quite satisfactorily treated with intermittent doses of chlorothiazide alone, there is equally little doubt that this form of treatment soon loses its effectiveness in the more severely affected patients. Treatment with spironolactone with or without dextroamphetamine and chlorothiazide has been continued for several months in some and for over a year in 1 of the more severely affected patients, with reduction of the edema in all of the patients. A dramatic response to spironolactone in 1 patient with severe edema encouraged the performance of subtotal adrenalectomy, and excellent results have followed in the 4 months elapsed since the operation.[87]

Toxemias of pregnancy. The urine of patients with pre-eclampsia has been shown to contain a great deal of aldosterone-like activity.[2, 12, 13, 94] However, recent work has shown that the output of aldosterone in toxemic patients, while frequently higher than that in nonpregnant controls, was usually *lower* than in pregnant women who had no signs of toxemia.[31, 45, 47, 61, 93] Moses, Lobotsky, and Lloyd[67] have shown that severe toxemia might occur in a woman previously submitted to total adrenalectomy for Cushing's syndrome who excreted only small amounts of aldosterone.

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*J. Conway and D. H. P. Streeten: Unpublished observations.

These observations cast considerable doubt on the earlier view that hyperaldosteronism might be etiologically related to the fluid accumulation in patients with toxemia. It is, therefore, not surprising that SC-8109 even in doses as high as 2 Gm. daily failed to ameliorate the fluid retention in 4 patients with toxemia of pregnancy.[1] The drug failed to induce natriuresis in the 1 toxemic patient whose data have been published in detail, whereas it produced a brisk increase in the urinary sodium excretion in a pregnant woman without toxemia.[1] Refractoriness of pre-eclamptic edema to therapy with spironolactone (400 to 800 mg. daily) has been observed also by Faloon.*

Toxic effects of the spirolactones

The toxic effects that have occurred in patients who receive spirolactone therapy have not been serious or life threatening. Drowsiness was common in patients given large doses of SC-8109[16] and occurs,[48] though far less frequently, in patients treated with spironolactone. Tenderness of the breasts has been seen in a female patient receiving spironolactone in large doses (1.2 Gm. daily), while mild hirsutism occurred in another woman after several months of treatment with 500 mg. of spironolactone daily.† The only other untoward reactions have consisted essentially of manifestations compatible with excessive degrees of aldosterone antagonism, such as hyperkalemia, hyponatremia, postural hypotension, and dizziness. Hyperkalemia is more likely to occur when potassium supplements are being administered concurrently. The tendency toward hyponatremia and postural hypotension may be increased by a reduced sodium intake or by exposure of the patient to high environmental temperatures, when excessive losses of sodium and water in the sweat probably result from antagonism of the increased aldosterone activity which is normally called forth

under these circumstances.[86] Hyponatremia probably also results from failure of the rate of water excretion to keep pace with urinary losses of sodium induced by the spironolactone. It is readily overcome by reducing the dosage of the drug or by administering a glucorticoid.[64]

Place of spironolactone in the therapy of edema

When the general condition of the patient is good and he or she has mild or moderate edema readily controlled by a thiazide or mercurial diuretic administered two or three times weekly, there appear to be no good reasons to use spironolactone in preference to the other diuretics. In two important circumstances, however, there is much to be gained from the use of spironolactone in the treatment of edema. First, spironolactone is usually effective in patients who have become refractory to continued treatment with other diuretics. In contrast to many of the commoner diuretics, spironolactone appears to retain its potency in the promotion of sodium excretion for many months or even for years, when used either alone or in combination with other diuretics. While its acute natriuretic potency is not comparable with that of the thiazide or mercurial diuretics, Morrison[64] has pointed out that there is sometimes more to be gained from a drug that will produce a slow but progressive loss of edema over several weeks than from an agent that acts more rapidly but loses its effectiveness when used daily.

Second, spironolactone neither increases urinary potassium excretion nor decreases the serum potassium concentration, as most other diuretics tend to do.[51] In fact, spironolactone will even reverse the tendency of the thiazide diuretics to induce potassium depletion and hypokalemia. Significant degrees of potassium deficiency are undeniably deleterious, since they not only predispose individuals to the arrhythmias (especially when digitalis preparations are being administered) but also probably contribute to the weakness, malaise, and gen-

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*W. W. Faloon: Personal communication.

†D. H. P. Streeten: Unpublished observations.

eral debility of patients with chronic ill health. The potassium-saving effects of spironolactone may account for the fact that improvement in strength and in sense of well-being seems to be more evident after therapy with this agent than after the use of other diuretics.

There is little doubt that spironolactone will prove to be a useful diuretic agent in the various types of edema described and may prove to be of value, too, in the management of hypertension and primary aldosteronism. Since the available evidence strongly favors the view that competitive antagonism of aldosterone is the main mechanism of action of spironolactone, the agent is also potentially a useful tool in the experimental and clinical investigation of the role of aldosterone in systemic disease.

Summary

1. Pharmacologic activity of the steroidal spirolactones apparently depends upon the presence of the 3-oxo-4-ene system in ring A and a five-membered lactone ring at the C-17 position.

2. The actions of the spirolactones on urinary excretion of Na^+, Cl^-, K^+, NH_3, titratable acid, and phosphate, as well as on salivary and fecal electrolytes, are the reverse of those of aldosterone and DCA and are not seen in untreated adrenal insufficiency. Since the secretion of aldosterone under most circumstances appears to be increased (and not decreased) by the compounds, it is probable that many of their actions result from antagonism of the effects of circulating aldosterone.

3. In general, there tends to be a lag between the onset of therapy with spirolactones and the diuretic effects. Diuresis is less profound but more enduring than that produced by thiazide or mercurial diuretics. When diuresis resulting from spirolactone administration is slight or absent, it may usually be initiated or greatly augmented by the additional use of a low sodium diet, potassium supplements, thiazide or mercurial diuretics, a glucocorticoid, and/or the intravenous infusion of

mannitol. Various combinations of these measures will maintain diuresis until edema fluid has disappeared in the great majority of patients, even when they have become refractory to any of the measures alone.

4. Spironolactone is a valuable new agent for the treatment of patients with cirrhosis and ascites who have become resistant to the common diuretic regimens. It is effective, too, in patients with the nephrotic syndrome, congestive heart failure, essential hypertension, primary aldosteronism, and the form of idiopathic edema that is associated with hyperaldosteronism, though its exact place vis-à-vis other drugs in the treatment of these disorders cannot yet be defined. At least it deserves therapeutic trial in many of these patients when an inadequate response is obtained to other means of treatment. The present evidence suggests that the agent is of little or no value in the treatment of the toxemias of pregnancy.

5. Toxic effects are not serious if the development of hyperkalemia, hyponatremia, or postural hypotension is promptly recognized and treated.

6. The chief advantages that will probably ensure a continuing place for the spirolactones or agents with similar properties in the treatment of the conditions listed are that these drugs usually maintain natriuretic potency for many weeks or almost indefinitely, they have a strong potentiating action on other diuretics to which the patient may have become refractory, and they tend to reduce rather than to increase potassium depletion and hypokalemia.

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Part VIII. Folic acid antagonists

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A review of folic acid antagonists is not a tale of ancient history, although natural deficiency of the vitamin must be timeless. Macrocytic anemia with diarrhea in India was early ascribed to dietary deficiency and a laboratory model sought. In 1919, monkeys fed only on autoclaved rice were observed to die, not with clinical signs of beriberi, but with weight loss, diarrhea, gastrointestinal ulceration, and anemia. Susceptibility to infection was recognized by frequently positive cardiac blood cultures at autopsy and the appearance of amebiasis during the deficiency state.[1] A description of the relief of tropical macrocytic anemia by large doses of autolyzed yeast† identified a source for the correcting factor.[2] Experimental confirmation of the effect of yeast in monkeys[3] was followed by extended study in other laboratories of the cause and cure (vitamin M) of a simian deficiency state which led to leukopenia, granulocytopenia, anemia, oral ulceration, diarrhea, and unusual susceptibility to infection, particularly bacillary dysentery.[4]

It was recognized independently that six of ten dogs fed a modified Goldberger diet developed, within 100 days, a syndrome of oral ulceration, leukopenia, maturation arrest of granulopoiesis, fever, sepsis, and death. This syndrome could also be reversed by yeast and by meat.[5]

Subsequently, production of yeast-correctible nutritional deficiencies in chicks (factor U)[6, 7] and recognition of growth factors from liver and yeast for *Lactobacillus casei* (norite eluate factor[8]) provided sensitive bioassays which led to isolation of folic acid from spinach,[9] eventual identification of the vitamin as pteryolglutamic acid,[10] recognition of its polyglutamate forms, and synthesis.[11]

The importance of folic acid in hematopoietic function provoked the interest of cancer investigators from the beginning. Dietary granulocytopenia in rats, caused by deficiency of folic acid, was corrected by administration of the vitamin.[12] Early attempts were made to treat neoplastic disease in mice with crude and purified vitamin and its polyglutamate conjugates.[13] The treatment of cancer in man with the first analogues of folic acid was not distinguished by impressive tumor regressions.[14-16] However, the substitution of an amino group for the hydroxyl group in the 4-position of the pteridine nucleus resulted in the synthesis of a far more powerful folic acid antagonist. Recognition of its remarkable inhibitory effects on microorganisms[17] and its effects in producing leukopenia in mice[18] was soon followed by a report of the production of temporary remissions in acute leukemia of children by Farber and his colleagues[16] in 1948.

Several prior reviews of folic acid and its antagonists have appeared.[19-29] This review will concentrate on the biochemistry of the folic acid vitamins as it provides understanding of the effects of antagonists, metabolic consequences of administering these compounds to man, the effects of folic acid antagonists in human diseases (choriocarcinoma, acute leukemia, other neoplastic diseases, psoriasis), effects in pregnancy, some studies of the regional use of amethopterin, and the effect of folic acid antagonists on some viral infections and immunity. No attempt has been made to include topics primarily pertinent to bacteriology, to present the extensive literature relating to animal tumors, or to consider all the folic acid antagonists which have been synthesized. No results in man seem superior to those obtained with 4-aminopteroylglutamic acid compounds, and the term folic acid antagonists used in this review is restricted to them.

Biochemistry

Studies of folic acid metabolism have recently reached the stage where information is available in chemical terms and on specific enzymatic activities, rather than solely in terms of growth responses of fastidious, yet deceivable, microorganisms. The folic acid vitamins are intimately involved in the pathways by which formate and formaldehyde participate in chemical reactions. The interrelations of the folic acid derivatives and the destinies of their one carbon fragments are shown diagrammatically in Fig. 1.

At the top of the diagram, the reduction of folic acid to dihydrofolic acid and then to tetrahydrofolic acid is shown. The cofactor function of the folic acid vitamins is served through the acquisition of the hydroxymethyl group from serine, and of formate, formaldehyde, and formimino radicals by tetrahydrofolic acid, and their subsequent donation to biosynthetic pathways.

The reduction of folic acid to tetrahydrofolic acid is catalyzed by folic reductase, an enzyme shown to be present in the liver of several species,[20, 30-34] in mammalian cells in culture,[35, 36] and in rodent intestinal mucosa.[37] Substantial evidence has been presented that the same enzyme is responsible for both reductive steps in chicken liver.[32] Enzymatic activities of human tissues and neoplasms are just becoming available. Reduced triphosphopyridine nucleotide is the hydrogen donor for reduction of folic acid in avian liver.[20, 30, 32]

Folic reductase is profoundly inhibited by 4-aminofolic acid* or amethopterin.† The affinity of the partially purified enzyme for 4-aminofolic acid is approximately 100,000 times greater than that for folic acid.[33] The strength of this affinity leads to nearly quantitative preferential saturation of the enzyme by 4-aminopteroylglutamic acid compounds, thus "pseudoirreversibly" excluding folic acid or dihydrofolic acid from the receptor site. This exclusion interrupts production of tetrahydrofolic acid and constitutes the main biochemical lesion caused by folic acid antagonists.[20] The high enzyme-drug affinity also explains the usual lack of reversing effect seen from concentrations of folic acid which are attainable at the enzyme site in vivo.[19, 33, 37]

The persistence of amethopterin in mouse liver for 3 weeks and more is consistent with continued enzyme-antagonist combination.[34] The return of enzymatic activity to liver and intestinal mucosa, despite the presence of retained drug in tissues, suggests that new enzyme is synthesized before the older enzyme-drug complex is eliminated.[37]

Four direct pathways are known in which a carbon atom becomes enzymatically attached to the N^5, N^{10}, or N^{5-10} position of tetrahydrofolic acid (Fig. 1). The four resulting compounds are all convertible to a fifth, N^{5-10} anhydroformyltetrahydrofolic acid. Interchange among the five forms, en-

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*Aminopterin.

†Methotrexate.
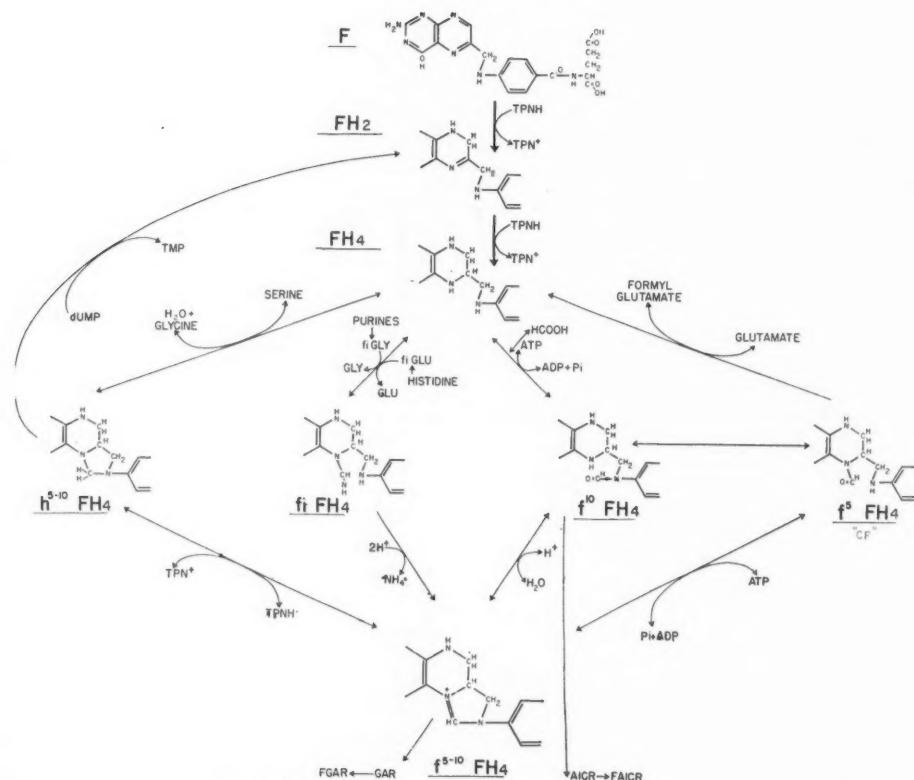
Fig. 1. Metabolic pathways of folic acid. The parts of the schematic formulas not shown are the same as for folic acid and do not change during the reactions illustrated. Those reactions known to be reversible are shown with double-headed arrows. *(F)* Folic acid; *(FH₂)* dihydrofolic acid; *(FH₄)* tetrahydrofolic acid; *(f)* a formyl group attached to the superscript numbered position; *(f⁵⁻¹⁰)* anhydroformyl (methenyl); *(h⁵⁻¹⁰)* hydroxymethyl (methylene); *(fi)* formimino; *(d UMP)* deoxyuridylic acid; *(TMP)* thymidylic acid; *(GLY)* glycine; *(GLU)* glutamic acid; *(FGAR)* formylglycineamide ribotide; *(FAICR)* formylaminoimidazolecarboxamide ribotide.

zymatically mediated by the pathways shown, is a dynamic requirement, since unique participation of single tetrahydrofolic cofactors in particular reactions has recently been demonstrated.[38]

The predominant quantitative end product after administration of C¹⁴-labeled formate is serine.[39] The circuitous route required for its synthesis is noteworthy (Fig. 1). The additional unequivocal destinies for formate and for other carbon atoms from tetrahydrofolic acid cofactors are formylglutamate,[40] the 2-position and 8-position of uric acid (and thus of the purine compounds from which uric acid is derived),[41, 42] and the 5-methyl group of thymidylic acid.[43] Each of these reactions has unique features. In addition, vitamin

B₁₂ serves as the prosthetic group for an enzyme from a mutant strain of *Escherichia coli* which catalyzes methionine synthesis from homocysteine and formaldehyde or serine. Optimal formation of the enzyme from vitamin B₁₂ and the apoenzyme requires tetrahydrofolic acid.[44] Other hydroxymethyl and methyl transfers may well prove to require the participation of folic acid vitamins in some analogous fashion.

Serine and glycine are freely interconvertible enzymatically in a reaction which involves N⁵⁻¹⁰ hydroxymethyltetrahydrofolic acid[45] (left, Fig. 1).* Folic acid antagonists diminish the serine yield from radio-

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*Physiologically, serine is probably the most important source for the formate and formaldehyde radicals of the one carbon pool.

active formate in incubation mixtures of circulating human chronic granulocytic leukemic cells[46] but not in mouse splenic lymphocytic leukemic L-1210 cells.[47]

N^{5-10} hydroxymethyltetrahydrofolic acid is involved as the donor for the 5-methyl position in thymidylic acid synthesis. Friedkin[48] has recently isolated tritiated thymidylic acid after incubation of a thymidylic acid–synthesizing system that employed tritiated tetrahydrofolic acid, formaldehyde as the carbon source, deoxyuridylic acid as the recipient, a partially purified enzyme from *E. coli,* and magnesium chloride. All the tritium was in the pyrimidine base, not in the sugar. The results support the concept that the hydroxymethyl group (from formaldehyde) was transferred from tetrahydrofolic acid to deoxyuridylic acid and that tetrahydrofolic acid itself served as a direct hydrogen donor (tritium in this experiment), thereby reducing the hydroxymethyl group to the methyl group of thymidylic acid. The products of the reaction were thymidylic acid and a folic acid compound at a lower level of hydrogenation than tetrahydrofolic acid, presumably dihydrofolic acid. It is of note that the subsequent reduction of dihydrofolic acid to tetrahydrofolic acid might be interrupted by inhibition of folic reductase with a folic acid antagonist, thus depleting the tetrahydrofolic acid pool.

The foregoing observations may be related to the evidence suggesting that thymidylic acid biosynthesis is the reaction most sensitive to the action of folic acid antagonists.[49-51] Amethopterin given to mice with line I lymphocytic leukemia inhibited the subsequent splenic incorporation of radioactive formate into thymine at a time when radioactivity actually increased in the purines measured.[49] Fifty-four per cent of radioactive formate incorporated during in vitro incubation of human chronic granulocytic leukemic leukocytes isolated from the peripheral blood was found in chromatographically isolated thymine. When amethopterin was added to the incubation mixture, formate incorpora-

tion into thymine was inhibited by 98.5 per cent. This contrasts with the 53 to 67 per cent inhibition of formate incorporation into purines in the ribonucleic acid or acid-soluble fractions.[51] It is not clear whether the pronounced inhibition of the synthetic pathway to thymidylic acid indicates need for higher cofactor concentration than in other reactions where tetrahydrofolic acid compounds act as cofactors.

Formiminoglycine has been shown to arise during the catabolism of purines by clostridial enzymes[52] (left center, Fig. 1). Formiminoglutamic acid has been found during mammalian catabolism of histidine.[53] After transfer to the tetrahydrofolic acid molecule, catalyzed by specific formiminotransferases, the imino group is removed as ammonia by the enzyme cyclodeaminase, with conversion of the remaining molecule to N^{5-10}-anhydroformyltetrahydrofolic acid.[54] Partially purified hepatic enzymes involved in the metabolism of formiminotetrahydrofolic acid are incompletely inhibited by 4-aminofolic acid. Formiminoglutamic formiminotransferase was inhibited 53 per cent, cyclodeaminase 49 per cent, and cyclohydrolase 60 per cent by 5×10^{-7} M 4-aminofolic acid. Lesser inhibition was obtained with amethopterin.[54] During intensive amethopterin administration to patients with neoplasms, only minute amounts of formiminoglutamic acid appear in the urine,[55] even after histidine challenge.[*]

Formate combines enzymatically with tetrahydrofolic acid to form N^{10}-formyltetrahydrofolic acid[56-58] (right center, Fig. 1). At physiologic pH, enzymatic and chemical equilibriums markedly favor this form of the cofactor over N^{5-10}-anhydroformyltetrahydrofolic acid.[52, 54, 59] Nonetheless, an active pathway from the N^{10}-formyl through the N^{5-10}-anhydroformyl (methenyl) to the N^{5-10}-hydroxymethyl (methylene) tetrahydrofolic acid is demonstrated by serine, which arises from administered

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[*] J. F. Holland and L. Mamrod: Unpublished observations.

formate as the predominant end product in some systems.[39, 56]

It has recently been established in purified enzyme systems that N^{10}-formyltetrahydrofolic acid supplies the carbon atom to 5-amino-4-imidazolecarboxamide ribotide to make 5-formylamino-4-imidazolecarboxamide ribotide. This atom becomes carbon 2 in inosinic acid (and in other purine compounds) after ring closure. N^{5-10}anhydroformyltetrahydrofolic acid provides the carbon atom to glycine amide ribotide to form formylglycineamide ribotide, which eventually becomes carbon 8 of inosinic acid (and other purine compounds). Although these donors appear to be specific, the interconversion of the two cofactors by the nearly ubiquitous enzyme cyclohydrolase and by rapid chemical equilibrium may deprive the cofactor specificity of much meaning in ordinary circumstances in vivo.[38]

The accumulation of 5-amino-4-imidazolecarboxamide ribotide, and not glycineamide ribotide, in extracts of *E. coli* and in sensitive L-1210 leukemic cells, when either is inhibited by amethopterin, suggests that a certain inhibitory specificity exists.[47, 60] Incorporation of carbon 2 into inosinic acid is more completely blocked than is that of carbon 8. This may reflect different competitive advantages of the drug for the two enzymes and their respective tetrahydrofolic acid cofactors.

N^5-formyltetrahydrofolic acid is the familiar citrovorum factor (CF) or folinic acid (right, Fig. 1). Microbiologic assays of tetrahydrofolic acid cofactors are described in terms of this component. Much of the N^5-formyltetrahydrofolic acid is formed from other unstable cofactors in the process of preparing and autoclaving specimens for bacterial assay.[61] Failure to preserve biologic fluids with a suitable reducing agent[62] and response of *Pediococcus cerevesiae* to thymidine present in the system[63] can lead, respectively, to low or high values in the citrovorum factor assay. Older conclusions drawn from bioassays require careful reappraisal. N^5-formyltetra-

hydrofolic acid can donate a formyl group to glutamic acid to become formylglutamic acid in a reversible reaction[40] and requires an energy-consuming reaction to become transformed to N^{5-10}-anhydroformyltetrahydrofolic acid.[64] Recently, direct conversion of N^5- to N^{10}-formyltetrahydrofolic acid without intermediate ring formation has also been shown.[65]

Although inhibition of folic reductase by folic acid antagonists seems to explain much of the action of these drugs, certain observations remain which are not readily understood unless some other substantial action of folic acid antagonists exists beyond the tetrahydrofolic acid level. Thus, when measured as cofactor activity for serine-glycine interconversion in an isolated system, $N\text{-}^{10}$-formyltetrahydrofolic acid was 50 per cent inhibited by a concentration of 4-aminofolic acid which inhibited tetrahydrofolic acid only 10 per cent.[45] Amethopterin inhibition of formate incorporation into chronic granulocytic leukemic leukocytes is partially reversible by citrovorum factor in a competitive manner.[66] Citrovorum factor served as an antagonist of amethopterin in mice over a drug range up to 100 times the LD_{50} for 5 days of administration with a nearly constant ratio of drug to vitamin, whereas folic acid was ineffective. Nonetheless, no effect of citrovorum factor was evident if it was administered more than 4 hours after a single supralethal dose of amethopterin.[67] To maintain 50 per cent inhibition of growth in L strain fibroblasts in culture, the ratio of citrovorum factor to amethopterin was constant over a two log range of concentration, suggesting that they were competitive antagonists.[68] Furthermore, differential accumulation of precursors in purine biosynthesis[47, 60] supports the existence of differences in drug effect on cofactor pathways beyond tetrahydrofolic acid.

The ultimate effects of folic antagonists in animals are highly tissue specific. The rapid rates of proliferation of the several epithelia of the alimentary canal appear to be correlated with indispensable require-

ments for folic acid cofactors. Inactivation of folic reductase by folic antagonists (and possibly other competitive actions) disrupts the capacity for cell multiplication.[37] This interference in biochemical reactions is manifest chiefly in those reactions known to be related to preparation for cell division, e.g., nucleic acid biosynthesis.[69] In a system where rapid turnover of cells is physiologic, such as alimentary mucosa or marrow, depletion of cell population follows with the clinical signs of ulceration or of leukopenia, thrombocytopenia, and erythrocytopenia.[28, 69a] Intracellular protein synthesis seems to be less significantly inhibited than nucleic acid synthesis. Erythrocyte precursors which remain become megaloblastoid and have increased amounts of cytoplasm,[70] cytoplasmic processes of epidermal epithelium (tonofibrils) become enlarged and prominent,[71] and C[14]-formate incorporation into serine is either uninhibited[47] (in mouse lymphocytic leukemic cells) or less affected than thymidylic acid biosynthesis[51] (in human chronic granulocytic leukemic cells).

In the preparations studied, the effects of folic acid antagonists on liver biochemistry in the mouse are confused by the frequent presence of leukemic cells in the liver and the effects of tumor metabolism on hepatic function. In normal DBA mouse liver, amethopterin caused no depression of formate incorporation into the combined protein and nucleic acid fraction.[39] This stands in contrast to nearly complete inhibition of folic reductase activity in liver after a single large dose of 4-aminofolic acid.[37] The liver is not a growing system, however, and the needs for folic cofactors for static, maintenance biochemical function seem to be vastly less demanding than those for cytogenesis.

One must also consider that the liver abounds in storage form(s) of folic acid, the exact nature of which is unknown. Polyglutamate peptides and perhaps other forms serve to make for a tissue-bound folic acid reservoir.[72] Despite the presence of 4-aminofolic acid, autolyzing sheep liver

produced 75 per cent of the expected citrovorum factor activity, suggesting that an antagonist-insensitive pathway can provide a folic cofactor.[73]

The effects of folic acid antagonists on neoplastic growth are not predictable from growth rates of either experimental or clinical tumors. Reliable prognostication of tumor suppression by biochemical indices currently available has been similarly unavailing. Drug effects on incorporation of formate into total proteins and nucleic acids in the gross protein fraction of leukocytes from patients with various hematologic disorders do not correlate with known clinical activities.[74] C[14]-formate incorporation into thymine of human chronic granulocytic leukemic leukocytes was inhibited by 98.5 per cent when incubated in vitro with amethopterin.[51] This activity of the drug is higher than in any other disease studied. It is not paralleled by clinical usefulness of the drug in chronic granulocytic leukemia, although slight antitumor effect can indeed be produced.[75]

Marked inhibition of formate incorporation into the gross nucleic acid and protein fraction of mouse leukemia L-1210[39, 47] is widely acknowledged, but attempts to identify the primary locus of activity, employing chromatographic separations, were beset by probable artifactual losses of thymine-containing compounds.[47] Of the compounds isolated, substantial accumulation of 5-amino-4-imidazolecarboxamide ribotide was found. Similar observations were made for a strain of leukemia L-1210 dependent on amethopterin for optimal growth; the same metabolic blockade was apparently present, but there was no evidence of its effectiveness in inhibiting tumor growth in the amethopterin-dependent tumor.[47]

Nucleic acid biosynthesis de novo, as measured by glycine-2-C[14] incorporation into adenine and guanine, was markedly inhibited by low doses of amethopterin in mouse TA3 ascites cell carcinoma. A concomitant slight increase in incorporation of preformed 8-C[14]-adenine may have been a

compensatory metabolic shift. An induced amethopterin-resistant line of the same tumor showed no inhibition of glycine utilization as a result of amethopterin treatment.[76]

Eighteen hours after 4-aminofolic acid treatment, the adenylic acid and adenosine triphosphate levels in 6-day-old sarcoma 180 mouse tumors were reduced to approximately 50 per cent of those of control animals. No effect was observed on levels of the compounds in liver, muscle, or spleen.[77]

Although studies of amethopterin effect on leukemic leukocyte metabolism, as reflected in rates of aerobic glycolysis, distinguish between acute lymphocytic leukemia and acute myelocytic leukemia in vitro,[78] the drug concentrations used in vitro are much greater than those attained by clinically effective doses. This casts doubt on the relevance of these observations to amethopterin function in vivo.

"Natural" resistance of most neoplasms to the effects of folic acid antagonists is poorly understood and has unfortunately received little study. Resistance to treatment has been observed to arise in several experimental neoplasms ordinarily sensitive to folic acid antagonists.[79, 80] Suspensions of splenic cells from mice carrying one of these amethopterin-resistant leukemias, when measured for ability to form citrovorum factor activity from added folic acid and serine, were less inhibited by antagonist than suspensions from the amethopterin-sensitive leukemia from which the resistant line derived.[81] Resistance may not be entirely a phenomenon of the tumor cells themselves, however, but many represent vagaries of tumor size, drug distribution, and metabolite availability in different tissues.[82, 83] Proof that one basis for the phenomenon of resistance can be genetic has been shown in bacteria never exposed to the drug. Treatment of *Diplococcus pneumoniae* with deoxyribonucleic acid derived from amethopterin-resistant *D. pneumoniae*[84] made the recipient organisms resistant without ever having been exposed to amethopterin.

Resistance to amethopterin has been induced in vitro by Fischer[85] in clonal strains derived from single cells of the cultured line of a murine lymphocytic leukemia, L-5178-Y. Stepwise increase or abrupt appearance of resistance has been produced, with a maximum resistance 100,000 times that of the original tumor. Cell lines which were four to 100 fold resistant to amethopterin in vitro were genetically stable, in the absence of drug, for the duration of the observation periods of 6 to 35 weeks. When transplanted back to mice, the 100,000 fold resistant line fades to a 20,000 fold resistant line in 4 weeks, suggesting that biochemical selection factors in vivo may tend toward a less resistant form of tumor cell. Fourfold resistance in culture was not detectable, in terms of therapeutic response, after implantation to mice. Fifty to 100 fold resistant strains in culture, however, were found to be resistant in mice. Other amethopterin-resistant L-5178 tumors in mice showed moderate drug resistance when cultured. These observations emphasize the possibility of studies on clinical material to determine drug sensitivities.

An examination of the mechanism of resistance has been made in sarcoma 180 cells gradually made resistant by prolonged growth in media containing amethopterin and folic acid supplemented with hypoxanthine (AH) or thymine (AT). The sixty-six fold AH and 160 fold AT resistant cell lines had greatly increased capacity to bind amethopterin in the cytoplasm as compared with the sensitive parent cells. These observations were explained by the isolation of increased amounts of folic acid reductase, the kinetic characteristics of which were unchanged, from the resistant strains. The folic reductase, aside from its quantity, was shown to be identical in the resistant lines with the enzyme present in the normal cell. Thus, resistance to amethopterin in these experiments would seem ascribable to sufficient folic reductase to fix the drug by binding, thereby physiologically inactivating it, together with

enough reductase to meet cellular requirements for tetrahydrofolic acid.[35]

A similar mechanism of resistance has been found for L-5178-Y lymphoblasts two- and sixteen fold resistant to amethopterin. The folic reductase content exactly paralleled their resistance. The enzyme was also apparently identical with normal folic reductase, since the same amounts of amethopterin were required to inhibit each unit of folic reductase activity in control and resistant strains. Because no other changes in folic acid metabolism were found, it was presumed that the folic reductase elevation constituted a single gene basis for resistance.[36]

Since prodigious levels of resistance can be elicited in cell cultures, it is manifest that the highest levels cannot be explained solely by increased content of folic reductase. The amount of binding required would exceed the possible cellular content of enzyme. At the level of resistance necessary to be recognizable as clinical refractoriness, however, enzymatic binding of drug in tumor cells could be an entirely sufficient explanation.

Circumvention of the effects of folic acid antagonists in mammalian cell cultures, similar to that noted in prior observations in bacterial systems, has been described.[86] Supplementation of Eagle's cell culture medium with thymidine, glycine, and hypoxanthine or adenine allowed for sustained growth in the presence of otherwise inhibitory levels of amethopterin. It is not inconceivable that similar nutrients might be available to neoplastic cells in vivo from the constituents of plasma and of necrosing normal cells at the advancing edge of, or from dying tumor cells within, a neoplasm. If so, lack of effect of amethopterin might be expected.

Recent studies have also shown two other biochemical effects which are difficult to associate with primary mechanisms of folic acid antagonist activity. Acetylation has been investigated using sulfanilamide as a convenient substrate. In a pigeon liver system, 99 per cent inhibition of

acetylation was found at 5×10^{-5} M amethopterin, approximately 10,000 times the concentration needed to inhibit folic reductase nearly quantitatively in isolated systems. In rabbits given sulfanilamide, higher serum acetylsulfanilamide concentrations were found in control animals than in those given 25 mg. of amethopterin per kilogram.[87]

Increased alkaline phosphatase activity in leukocytes was noted when incubations in vitro were conducted in the presence of 25 to 100 γ of amethopterin per milliliter. This effect was not reversible by preaddition of folic acid, citrovorum factor, or vitamin B_{12}. If citrovorum factor was added simultaneously with amethopterin, however, the increase in phosphatase activity was mitigated.[88]

Biochemical, pharmacologic, and toxicologic effects in man

Observations in rats with intestinal bacteriostasis fed folic acid deficient diets disclosed a urinary component containing glutamic acid.[89] The material has been identified as formiminoglutamic acid (FGA), a catabolite of histidine after ring cleavage[53] (see Fig. 1). Early examination of FGA by microbiologic assay disclosed its accumulation in the urine of patients (and normal subjects) treated with amethopterin presumably because of depletion of available tetrahydrofolic acid.[90, 91] A much preferable enzymatic assay dependent on the reactions cited above has been described[92] and used to study normal subjects and children with acute leukemia during amethopterin administration.[93]

The excretion rate of FGA in 23 subjects, including normal adults and adults and children with neoplasms and other diseases, varied from zero to 0.51 μM per hour. (If the excretion rate were constant, 1 μM per hour would equal 4.18 mg. per day; actually the rate is not constant.) In 3 children with acute leukemia who had not previously had amethopterin treatment, the FGA excretion rate was 0 to 0.03 μM per hour. In each of 16 children with acute

leukemia who received amethopterin therapy, FGA excretion at some time was 1.30 μM per hour or greater. Many weeks passed in some children, as many as 15 weeks in 1 who also received prednisone, before substantial increase in the FGA excretion rate occurred. In 2 children treated with amethopterin alone, normal excretion rates for FGA shortly after a remission of acute leukemia was established were interpreted by the authors as evidence that the leukemic cells were more sensitive to the folic acid antagonist than was the pathway responsible for formiminoglutamic aciduria. Patients with drug intoxication were not studied, and only 1 boy in transition from frank leukemia to remission status was observed. He had an abnormally high rate of FGA excretion at 14 days.[93]

We have studied FGA excretion with and without histidine loading during a study of amethopterin and dichloramethopterin treatment for lymphosarcoma.[*] Urinary FGA on a regular diet and on regular diet plus a single oral dose of 5 Gm. of histidine was determined once weekly. The increased excretion of FGA after a histidine challenge in 7 normal adults varied from 13 to 66 μM per day. In some patients taking a folic acid antagonist, the increase after histidine challenge[93a] did not exceed the values in the normal subjects. When compared to an individual patient's pretreatment control values, however, FGA excretion after histidine challenge serves to identify a biochemical lesion which appears within a few weeks of folic acid antagonist administration. Rise in FGA excretion without histidine loading, if recognized at all, always occured later than the response to histidine challenge. Several patients showed clinical toxic effects during drug administration, as evidenced by oral mucosal ulceration or hematopoietic change, but in our studies neither morphologic evidence of intoxication nor tumor regression was successfully predicted by changes in

FGA excretion, with or without histidine challenge. It has recently been shown that vitamin B_{12} and several sulfur-containing amino acids can correct formiminoglutamic aciduria in folic acid deficient rats.[94] The effects of these substances in folic acid antagonist–induced formiminoglutamic aciduria in man are unknown.

Urinary formic acid excretion has recently been enzymatically measured as a parameter of drug-induced folic acid deficiency,[95] since urinary formate is known to rise in folic acid deficient rats.[96] The excretion rate in normal adults and in adult patients with cancer and other diseases was 3.6 to 47.3 μM per hour. In children with leukemia or other diseases who had never had amethopterin, the excretion rate was 1.5 to 15.7 μM per hour. Fifteen of 16 children who received amethopterin excreted more than 15.7 μM of formic acid per hour. Delays in increase of excretion rate were as long as 6 weeks in a child receiving amethopterin alone and 12 weeks in a child also receiving prednisone. In two instances of children taking only amethopterin, remission of the leukemia was established before increase of formaturia occurred. Since the study involved the same patients (and specimens) as those in whom formiminoglutamic acid excretion rates had been determined,[93] comparisons of the excretion of these two urinary metabolites could be made. It is of interest that in only one instance of thirty-four specimens in 16 patients were the directions of change discordant. Urinary formate can arise from several known[97] and perhaps from unknown precursors. The effect of diet and factors others than folic acid antagonism on the urinary excretion of formate in man has not been delineated.

Impaired hydroxylation of phenylalanine to tyrosine has been observed in women under treatment with amethopterin for choriocarcinoma. As early as 12 hours after amethopterin administration, a loading dose of phenylalanine led to higher levels and delayed disappearance of phenylalanine from serum and to a lower serum tyro-

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[*]J. F. Holland and L. Mamrod: Unpublished observations.

sine concentration than had occurred after a similar challenge before amethopterin treatment. Basal phenylalanine and tyrosine serum levels were unaffected.[98] These findings are clinical extrapolations of the observations of Kaufman[99, 100] that enzymatic hydroxylation of phenylalanine requires an unidentified cofactor (for which synthetic tetrahydropteridines or tetrahydrofolic acid can substitute). There is evidence that the cofactor requires reduction before exhibiting activity, in a fashion analogous to the reduction of folic acid to tetrahydrofolic acid. This reduction is effectively blocked by amethopterin, leading to impaired hydroxylation of phenylalanine to tyrosine.

Decrease in urinary corticosteroid excretion has been described after 4-aminofolic acid administration in some patients,[101] but the metabolic effects and antineoplastic actions of folic acid antagonists are not considered to be mediated through the adrenal.

Urinary citrovorum factor activity has recently been assayed with *Ped. cerevisiae* 8081 resistant to amethopterin.[102] On days when large doses of amethopterin were administered, citrovorum factor activity increased three to five times over control levels. This increase was not correlated with therapeutic response and thus did not reflect tumor tissue destruction. The relatively minor increase in urinary nitrogen excretion on days of drug administration suggests that the citrovorum activity did not come from destruction of normal tissue. Presumably, it represented displacement of tetrahydrofolic acid cofactors from enzymatic sites. These data extend and confirm earlier studies in the rat.[103]

Several days after 4-aminofolic acid administration, 92 per cent of administered folic acid was recovered as folic acid microbiologically, in contrast to 68 per cent under control circumstances. This finding was attributed to a decrease in extrarenal removal of folic acid, its mobilization from an extrarenal site (at least in part liver), and decreased renal tubular reabsorption of the filtered folic acid load.[62]

The effects of amethopterin on metabolism were observed in classic metabolic balance studies in 3 women with minimal disease from metastatic choriocarcinoma. Each had had multiple prior courses of amethopterin.[104] None showed change in tumor size or decrease of gonadotropic titer as evidence of tumor suppression during the study. A single 5 day control period, 5 to 15 days after the last administration of amethopterin, was followed by 5 days of treatment during which 25 mg. of amethopterin was given intramuscularly daily and by another 5 days of no treatment. Although changes were small, they were consistent for these 3 patients. A decrease of 3, 3.5, and 3.5 Gm., respectively, occurred in over-all nitrogen balance for the treatment period when contrasted with the control. The extra nitrogen loss in the 3 women was followed by a more positive nitrogen balance in the posttreatment period, suggesting an anabolic phase after the catabolic episode.

Li and co-workers[102] have studied, under conditions of constant intake, a man with choriocarcinoma who also failed to improve with amethopterin. Urinary nitrogen excretion seemed to increase by up to 1 Gm. daily on the days he received amethopterin orally.

An increased average daily urinary loss of uric acid of 58, 382, and 104 mg., respectively, was observed during amethopterin administration in the 3 aforementioned women.[104] The nitrogen and urate loss during amethopterin administration was temporally related to a consistent decrease in reticulocyte count. The authors estimated that red cell production virtually ceased in their patients during amethopterin treatment and that the changes in nitrogen balance, assuming continuing normal erythrocyte catabolism, might be ascribed to failure of reutilization of erythrocyte catabolites because of the drug effect on marrow. Unfortunately, data on other marrow constituents were not presented, but it is entirely possible that concomitant effects on leukopoiesis might

have interrupted normal marrow production of leukocytes. Although one might anticipate decreased urate excretion on the basis of the locus of action of amethopterin on purine biosynthesis,[75] the diversion of preformed purine-containing compounds to produce increased urinary uric acid could readily be explained if substantial amounts of these compounds were not used in hematopoiesis.[104] The phenomenon of increased urate excretion has been observed in acute leukemia after amethopterin administration.[105, 106]

A metabolic study was also performed in a woman with choriocarcinoma not previously treated with amethopterin.[104] A drop in chorionic gonadotropic titer during administration suggests that at least dysfunction and perhaps growth inhibition of occult tumor occurred. The nitrogen balance became more positive by 6.1 Gm. in the 5 day treatment period without change in phosphorus balance. These findings are not incompatible with results seen in anabolism of host tissues after chemotherapeutic tumor destruction.[107]

When measured microbiologically, amethopterin is rapidly absorbed after ingestion and reaches peak serum levels in ½ hour in fasting subjects. The peak serum concentration is lower and occurs as late as 3 hours if the drug is taken after eating. Approximately one-half the microbiologic inhibitory activity of administered amethopterin is recovered in the urine at 24 hours. Renal functional impairment greatly prolongs and elevates serum concentrations and provides ample explanation for the enhanced toxicity of folic acid antagonists in patients with renal insufficiency.[108]

When measured fluorometrically,[109] the serum level of amethopterin 1 hour after an oral dose was approximately twice that measured by the microbiologic technique after the same dose.[108] At serum concentrations of the drug in the ordinary range, 43 to 47 per cent was found by equilibrium dialysis to be protein bound. Freeman, using the fluorometric technique, stated that 85 to 100 per cent of the orally admin-

istered drug was recovered in the urine by 12 hours. After intravenous administration, a similar nearly quantitative recovery occurred by 6 hours.[110]

It is of note that no drug was discovered in human liver at autopsy 18 to 120 days after prolonged clinical courses of amethopterin.[110] It is not certain whether the inconsistency between this finding and the demonstration of prolonged hepatic binding of the drug in mice[33, 34] is methodologic or the result of a striking species difference. The volume of distribution of amethopterin is 18 to 29 per cent of total body water, suggesting that the distribution is primarily extracellular.[110] Study of metabolites of amethopterin by column chromatographic separation of urine failed to reveal any reduction of amethopterin to the di or tetrahydro form.[111]

Recently a specific technique for measurement of folic acid antagonists has been devised. This method depends on the intense affinity of folic acid antagonists for folic reductase. The enzyme, derived from the supernatant obtained by high speed centrifugation of rat liver homogenate, is used as a reactant to bind drug; excess enzyme is determined by its activity in reducing folic acid to tetrahydrofolic acid. Comparison with a curve obtained with standard amounts of drug allows precise measurement.[33] The specificity of this method has given more reliable data than microbiologic or fluorometric determinations.

The morphologic effects of folic acid antagonists in man are most prominently seen on alimentary mucous membrane (described in the section on leukemia), hematopoiesis, hair growth, skin, spermatogenesis, and the growth of certain tumors. After single large doses of amethopterin intravenously in 10 cancer patients without hematologic disorder, Condit found that maximum reticulocytopenia usually occurred on the fourth to fifth day, neutropenia on the sixth day, and thrombocytopenia on the ninth day. These times reasonably approximate the lifespans for

the particular cell types when measured by other techniques. The data suggest that the effect of amethopterin is to interfere with marrow production of these cells. Lymphocytopenia was not observed in these experiments.[111a]

Megablastoid changes in erythropoietic cells are commonly seen in patients who receive amethopterin for an extended period. Iron uptake measured by Fe^{59} continues about normally for 7 to 10 hours after an injection of amethopterin and then slows. This is consistent with an effect of folic acid antagonists on a stage in erythropoiesis earlier than iron incorporation. Cells which have already matured sufficiently at the time of drug administration continue iron metabolism normally.[111b] Larger amounts of drug lead to profound pancytopenia, but rarely without significant toxic effect on the alimentary mucosa. "Marrow overshoot" with brisk leukocytosis and thrombocytosis may occur in the recovery phase following a bout of hematologic toxic effect.

Amethopterin exerts profound effect on actively growing (anagenic) hair. Alopecia may occur during treatment but is usually not prominent. Van Scott, Reinertson, and Steinmuller[112] have described a constriction of the shaft distal to the keratogenous zone recognizable under a dissecting microscope; the hairs break at this segment. The findings are different from atrophy of the hair bulb induced by x-ray. Morphologic changes in the hair shaft have been detected as early as the eighth day and in all patients studied by the fourteenth day after large single injections of amethopterin. Changes also occur in hair of patients who receive standard doses of the drug.

Amethopterin and 4-aminofolic acid induce transient oligospermia. In 4 patients, 2 on each drug, total sperm counts were depressed 63 to 97 per cent below control levels. The two men on 4-aminofolic acid were psoriatic patients who had been treated intermittently for 2 and 3 years. The sperm counts between courses were normal, however, indicating that permanent oligospermia had not occurred.[71]

Chemotherapy of trophoblastic neoplasms

Perhaps the most significant clinical achievements of amethopterin occur in the chemotherapy of trophoblastic neoplasms of women. The detailed, brilliant observations of Li, Hertz, and Spencer,[113] initially after intravenous administration of high doses in the course of pharmacologic studies by Condit[*][69a, 113] and subsequently after modification to another more protracted regimen of intensive administration, clearly delineated this important use of the drug.

Appropriate classification of trophoblastic neoplasms is indispensable to an appraisal of the effects of treatment.[114] Choriocarcinoma in women is a highly malignant tumor which usually arises from placental tissue. It is distinguished by its morphologic disorganization (lack of villi) and extreme invasiveness. Although surgical cures are reported, the ordinary tragic course is characterized by early metastasis, rapid growth, and death within a year from diagnosis. Spontaneous remission of histologically confirmed choriocarcinoma is rare. Invasive hydatidiform mole (choriocarcinoma destruens) is a more benign neoplasm than choriocarcinoma. The molar tissue, characterized by hydropic "degeneration" of villi, invades the uterine wall and may metastasize distantly. The morphologic state of metastases from invasive mole, however, may be either choriocarcinomatous or molar. Invasive mole has an appreciable spontaneous regression rate. Since the term chorionepithelioma has been commonly used to describe both choriocarcinoma and invasive mole, it is likely that most of the spontaneous regressions reported in the world literature for chorionepithelioma were in fact of invasive mole. Hydatidiform mole is, by definition, confined to the endometrium and uterine cavity and does not require chemotherapy.

Chorionic neoplasms secrete chorionic gonadotropin, a hormone which may be detected in urine and serum and which can

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*P. T. Condit: Personal communication, 1959.

be quantified by bioassay. Its secretion provides a convenient gauge of at least one function of the neoplasm.

The intensive regimen of amethopterin treatment advocated by Hertz and co-workers[115] comprises courses of treatment of 5 days' duration in which 10 to 30 mg. is given daily, usually intramuscularly. In some patients, oral or intravenous administration has also been used. To our own patients,[114] amethopterin has been administered orally in courses of 4 to 5 days at total dose levels of 0.75 to 1.5 mg. per kilogram in each course. Repeated courses of the drug have been administered in the presence of detectable tumor, 10 to 14 days after the last course, if all signs of toxic effect from the compound have abated.

Amethopterin administration in choriocarcinoma or invasive mole can cause sharp decline in the titer of urinary gonadotropin in a matter of days, but three courses of drug may be necessary before any decline in titer begins.[116] Several courses of drug may be required before excretion of chorionic gonadotropin falls to normal levels and remains there.[113, 114, 117, 118] In patients with fever from metastatic choriocarcinoma, defervescence may follow directly

upon the initiation of amethopterin.[114] A sense of well-being may also be recognizable before clinical evidence of a change in tumor mass.

Decrease in the size of tumor metastases usually accompanies laboratory and clinical indications of improvement. Since pulmonary metastases from choriocarcinoma are frequently present, serial x-ray films serve to demonstrate the beginning regression of metastases, even those several centimeters in diameter, which is noticeable as early as the second week.[118] Some regressions are partial.

Complete remission of choriocarcinoma has been defined as absence of all hormonal, radiologic, and physical evidence of tumor. Partial remission has been characterized by substantial decrease in gonadotropin excretion and decrease or disappearance of gross tumor. In addition, the term partial remission is applied to the remission phase of those patients who had sustained regression of tumor, fall in titer, and clinical improvement but who are known subsequently to have relapsed.

Recently, there has been a review of 47 women with trophoblastic neoplasms treated by an intensive amethopterin regimen; the patients were reported from eight different clinics as of February, 1960.[114] Table I summarizes the pertinent data. The complete remission rate of choriocarcinoma is less than that of invasive mole. This is in keeping with the greater responsiveness of the latter neoplasm to several other chemotherapeutic agents. No other drug than amethopterin has effected a similar complete remission rate in patients with choriocarcinoma.[114] Since the treatment of patients with choriocarcinoma started only in March, 1956, however, and since only 3 reported patients with choriocarcinoma can possibly be surviving more than 40 months in March, 1960 (patients 1 and 6 of Hertz and colleagues[115] and patient 2 of Hreshchyshyn, Graham, and Holland[114]), it is premature to talk in terms of cure.

Failure of amethopterin in choriocarcinoma has been prominent in patients with

Table I. *Treatment of choriocarcinoma, invasive mole, and other trophoblastic neoplasms in women with intensive amethopterin therapy**

Disease	No. of patients	Complete remission	Partial remission	Failure
Choriocarcinoma	36	10	17	9
Invasive mole (chorioadenoma destruens)	6	4	2	0
Other trophoblastic neoplasms†	5	1	4	0

*Patients of Hertz and colleagues,[115] Li,[116] Douglas,[119] Doniach, Crookston, and Cope,[120] Vanaas,[121] Buckle,[122] Perlson and Whitsitt,[123] and Hreshchyshyn, Graham, and Holland.[114]

†Malignant chorionepithelioma (1), probable choriocarcinoma (2), trophoblastic nodule of cervix (1), and inconclusive diagnosis (1).

massive amounts of metastatic tumor when first treated.[114, 115] The continuing elevation of gonadotropin excretion, which consigns one-half the women with trophoblastic neoplasms to the partial remission category, doubtless reflects persistent functional tumor tissue in some site not determinable by physical or roentgenologic examination. Persistence, recurrence, or appearance of intracranial metastases during treatment has been reported[115] and may constitute an occult tumor source of increased gonadotropin excretion. Since the possibility of eradication of choriocarcinoma and other trophoblastic neoplasms with suitable therapy is now real, however, every effort should be made, in the event of failure to attain complete remission from drug, to assure that a resectable mass of tumor is not the producing site. In 1 of our patients (patient 7[114]), gonadotropin excretion finally reached a normal titer 7 months after initiation of a chemotherapeutic and surgical program for the management of choriocarcinoma.

The occurrence of toxic effects of amethopterin in seriously ill patients with the high doses of drug used is not surprising. After patients have reached remission status, identical doses can often be tolerated with minimal or no toxicity.[114] Of the 47 reported women with trophoblastic neoplasms treated with amethopterin in nearly 300 courses (Table I), in only 1 was amethopterin toxicity thought to be the chief cause of death, although toxic lesions were seen in 2 other patients at autopsy.[115]

The response of invasive mole to amethopterin is not better than results reported for mechlorethamine oxide hydrochloride.*[114] The greater over-all experience that has accumulated with amethopterin, however, would seem to make it the drug of choice at present.

Failures of nitrogen mustard,[114] DON (6-diazo-5-oxo-L-norleucine),[114] 6-mercaptopurine,[117] and thio-TEPA (triethylenethiophosphoramide)[114] to improve patients with choriocarcinoma have been reported. A single patient has responded to a combination of actinomycin D and chlorambucil after relapse from amethopterin[124]; the subsequent complete remission was still in progress 8 months after onset.* One patient who failed to benefit from three oral courses of amethopterin responded to parenteral dichloramethopterin.[114] Recently, Hertz, Lipsett, and May[125] have introduced the use of vincaleukoblastine, an alkaloid from periwinkle, in women who had become resistant to amethopterin. Three of 8 patients had a return of gonadotropic excretion to normal levels, in 1 instance for 5 months. Partial regression of pulmonary metastases was described in 2 other patients. The mechanism of action of this compound is unknown but apparently is not similar to that of amethopterin.

Choriocarcinoma in men has not responded satisfactorily to amethopterin treatment.[117] In 4 men with choriocarcinoma of testicular origin, three to eight courses of amethopterin and two to four courses of intensive 6-mercaptopurine treatment failed to diminish gonadotropin titer significantly, and death from pulmonary insufficiency occurred in each. In a fifth man, choriocarcinoma from an unknown primary site failed to respond to seven courses of amethopterin, and he died in similar manner.

Recently, Li and co-workers* have devised a combination chemotherapy regimen which evoked some evidence of objective response in 9 of 17 men with metastatic testicular choriocarcinoma for periods of 3 to 40 months. This regimen consists of amethopterin, chlorambucil, and actinomycin D given concurrently. The activities of each component, their essentiality for the responses seen, and the completeness of the remissions induced have not yet been defined.

Recently, Li has investigated the transplantability of choriocarcinoma from women and from men.* He has implanted

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*Nitromin.

*M. C. Li: Personal communication, 1959.

choriocarcinoma of testicular or uterine origin into cheek pouches of hamsters maintained on diets with and without folic acid supplementation in the form of the crystalline vitamin or of spinach. Preliminary results of his studies on 196 animals indicate that choriocarcinoma of uterine origin implants poorly (14 per cent) unless added folic acid with or without corticosteroids is provided (46 per cent to 60 per cent). Testicular choriocarcinoma implants well in steroid-treated hamsters (65 per cent) and can grow even in animals receiving no spinach. The findings are not inconsistent with the concept that critical metabolic needs for folic acid vitamins in choriocarcinoma of the female provide a basis for sensitivity to folic acid antagonists. Direct measurements of vitamin content and folic reductase will be of particular interest in choriocarcinomas of men and women.

Folic acid antagonists and pregnancy

The use of folic acid antagonists to induce abortion rests on their greater toxicity for fetal than maternal tissues in pregnant mice and rats[126] and their activity in some women for whom therapeutic abortion has been attempted.[127] Recent reports from several countries of severe maternal intoxication,[128-130] of fetal anomalies,[127, 131, 132] and of inefficacy in six of twelve attempted therapeutic abortions[128] probably reflect a small portion of the difficulties encountered and a smaller proportion of the attempts made. One must conclude that the technique is uncertain and unsafe and has two serious drawbacks not shared with even surreptitious curettage: fetal survival with anomalies and attempted suicide with the abortifacient. There would appear to be no reliable basis, either in medical practice or in the demimonde, for currently available folic acid antagonists to replace curettage in terminating early pregnancy. The therapeutic use of folic acid antagonists during pregnancy, at least during the first trimester, must be scrupulously avoided.[133] In women in whom pregnancy might start during administration of folic acid antagonists, the physician must reckon with the possibility of discovering the pregnancy after fetal damage has occurred.

Chemotherapy of acute leukemia

The use of 4-aminofolic acid antagonists in acute leukemia of childhood started with the initial patients treated with 4-aminofolic acid by Farber and his associates in 1947.[16] Amethopterin has virtually displaced 4-aminofolic acid in clinical usage and still constitutes, together with corticosteroids and 6-mercaptopurine, the established therapy for acute leukemia in children. The results of treatment with the drug in acute leukemia of adults have been grossly disappointing. Several informative reviews of this general area are available.[28, 134-136]

The data on patients from nineteen presentations at a symposium on folic acid antagonists[137] have been assembled by this reviewer. Of 731 children, 55 per cent were considered to have been benefited. If, as has been common practice in some clinics, one excludes all patients who did not receive 21 days of drug treatment, 627 children remain, of whom 34 per cent had complete remissions as judged by the reporting investigator, 20 per cent had partial remissions, and 16 per cent had a lesser degree of improvement. Thus, 70 per cent of children sustained some recognizable benefit. In some patients, the contributions of corticotropin and corticosteroids to the clinical and hematologic improvement could not be estimated.[137] Of 163 adults discussed by the same authors, only 14 per cent had any evidence of improvement at all. A survey of the world literature conducted by Sampey disclosed nearly 3,000 patients with acute leukemia treated with folic acid antagonists. Of these, 47 per cent were considered to have sustained a remission.[138]

Since the time of the clinical introduction of folic antagonists, 6-mercaptopurine

has been found to be an effective drug, even in patients in whom acute leukemia is unresponsive or no longer responsive to folic acid antagonists.[139] A concurrent controlled study of these two compounds was undertaken to assess in man some of the chemotherapeutic principles that have been developed in animals with responsive tumors.

A study group of several cooperating investigators placed all their untreated patients with acute leukemia in one of three treatment programs at random.* Drugs were administered orally in doses determined by age and weight. The first program provided for the administration of amethopterin for 6 weeks; an observation period of 2 weeks was followed by 6 weeks of treatment with 6-mercaptopurine. If therapeutic response or frankly progressive leukemia was evident during any phase of the study, prolongation or curtailment of that phase was accomplished by a uniform protocol used by all investigators. The second program was identical with the first, except that 6-mercaptopurine was administered first and followed by amethopterin. The third program consisted of the administration of amethopterin and 6-mercaptopurine in full doses in combination for a single phase of the study. Of the 285 patients whose records were accepted for study, there were 128 patients less than 20 years of age (children) with acute lymphocytic leukemia and 96 adults older than 20 years with acute myelocytic leukemia.

In the acute lymphocytic leukemia group, 48 children were treated with amethopterin. Complete remissions (as rigorously defined[140]) occurred in 22 per cent. Other details of remission status are given in Table II. The role of corticosteroids in bringing about a large part of the re-

missions cannot be assessed, and these instances are therefore presented separately. The 41 survivors of the amethopterin phase who were treated with 6-mercaptopurine sustained a more favorable remission experience, if the steroid cases are discounted.

In the second program, 43 children were treated with 6-mercaptopurine first. The 33 survivors who were then given amethopterin did not differ significantly from those in the first program to whom amethopterin was given as the first drug. Neither did amethopterin as a second treatment after 6-mercaptopurine compare favorably with 6-mercaptopurine as a second treatment following amethopterin. The groups are small, however, and the evidence must be regarded at present as suggestive only.

Administration of the drugs in combination was not inferior in terms of either complete remissions produced (when contrasted to both phases of the sequential programs) or the total remission experience.

The study in children failed to provide evidence for the operation of collateral sensitivity. The median duration of complete remissions was the same for all treatment programs, but the longest remissions were seen in the simultaneous combination group, suggesting that combined treatment had some merit.

The results of chemotherapy of adults with acute myelocytic leukemia are decidedly inferior to the experience with acute lymphocytic leukemia in the young. (Table III.)

Certainly, amethopterin is barely active in adult acute myelocytic leukemia, where only one complete and one partial remission occurred in forty total courses. It should be appreciated, too, that only seven of forty-three courses of 6-mercaptopurine in adults with acute myelocytic leukemia resulted in any remission status, and in two of these, the role of corticosteroids is uncertain.

Conclusions from this study must await more searching analysis than provided here, particularly with respect to pro-

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*Acute Leukemia Study Group B, Clinical Studies Panel, CCNSC, E. Frei, III, Chairman: A study of sequential and combination chemotherapy with antimetabolites (Methotrexate and 6-mercaptopurine) in human acute leukemia. Observations, 1960; submitted for publication.

Table II. *Rates of response to treatment of acute lymphocytic leukemia in patients less than 20 years of age*

Program	No. of patients	Complete remission (%)	Remission with corticosteroids (%)	Partial remission (%)	Total remission status (%)
First					
Amethopterin	48	22	26	7	55
6-Mercaptopurine	41	24	11	20	55
Second					
6-Mercaptopurine	43	26	14	21	61
Amethopterin	33	18	9	6	33
Third					
Amethopterin plus 6-Mercaptopurine	39	44	8	15	67

Patients were divided into the three programs at random; see text.

Data from Acute Leukemia Study Group B, Clinical Studies Panel, CCNSC, E. Frei, III, Chairman: A study of sequential and combination chemotherapy with antimetabolites (Methotrexate and 6-mercaptopurine) in acute leukemia. Observations, 1960; submitted for publication.

longation of life, concomitant toxicity, and comparability of steroid use.*

The effect of folic acid antagonists on survival in acute lymphocytic leukemia has been considered by Haut and associates[141, 142] in the course of analysis of the effects of chemotherapy. Of 101 patients of all age groups seen in a 10¾ year period, the median survival from onset of treatment of 21 patients given corticosteroids followed by folic acid antagonists was 6 months. This was significantly shorter (p = 0.02) than for 46 patients who received corticosteroids followed by 6-mercaptopurine at some time and often by an additional drug. The median survival for these 46 was 9 months. Groups of patients who received only two drugs, of which one was either amethopterin or 6-mercaptopurine, were then analyzed, but no difference in survival was found that would point to a superiority of either antimetabolite. The authors concluded that the significant factor in prolongation of survival in lymphoblastic leukemia appears to be the number

of "effective" drugs used seriatim rather than the use of any one in particular. This, of course, is only true when the drug activities are marginally effective. A remarkably effective folic acid antagonist or other drug should have a recognizable individual contribution to extension of survival.

The effects of folic acid antagonists in patients with myeloblastic leukemia were not reflected in increased survival.[142]

The usual administration of amethopterin to children with acute leukemia is accomplished on a daily oral schedule. Fractionation of the dose resulting from failure to warn the child's mother against this practice can lead to early intoxication. The usual daily dose of 2.5 to 5 mg. may be increased in larger individuals and decreased in infants. An approximate level of 0.1 mg. per kilogram per day is a reasonable guide if physiologic evidence of drug effect has not yet appeared. Beginning evidence of drug effect, e.g., falling leukocyte count or decreasing node or organ size, may occur within the first few days. In other patients, antileukemic action may become obvious only after a month or more of no apparent clinical change. It may be particularly difficult to see early effects in patients who had leuko-

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*Acute Leukemia Study Group B. Clinical Studies Panel, CCNSC, E. Frei, III, Chairman: A study of sequential and combination chemotherapy with antimetabolites (Methotrexate and 6-mercaptopurine) in human acute leukemia. Observations, 1960; submitted for publication.

penia at the outset. An initial rise in circulating platelet count and neutrophil and reticulocyte percentage in the peripheral blood is evidence of regenerating normal marrow function. Marrow aspirations performed at, or just before, these peripheral indications usually reveal decrease or disappearance of leukemic cells and reappearance of normal hematopoietic cells.

Toxic effects from the drug, or clinical phenomena difficult to distinguish from them, are not infrequent in the course of treating leukemic children not in remission. The healthier child in remission can often tolerate, as a maintenance dose, a level which induced toxic effects during the active leukemic stage.

Toxic effects of the drug in acute leukemia are the same as in other diseases, except for the much greater difficulty in differential diagnosis from manifestations of the leukemia itself. Chronic hepatic disease in leukemic children maintained for long periods on the drug has been described[143] but may be a manifestation of the leukemia or of other treatments. This type of postnecrotic cirrhosis has not been described in other patients given folic acid antagonists.

Oral lesions of folic acid antagonist intoxication are often difficult to distinguish with certainty from oral lesions which occur in leukemia. Ulcerations on the lingual or buccal mucosa, 2 to 3 mm. in diameter with a red or gray shaggy base and surrounding erythema, are rather typical of folic acid antagonist intoxication. Scaly lips, cracks at the angles of the mouth, necrotic slough of the interdental papillae, and necrosis of a single papilla on the tongue may all be toxic manifestations too, but must be differentiated from lip picking and dryness, leukemic infiltration of the gums, and ulcerated petechial hemorrhages in the submucosa. Pharyngitis and boggy edematous buccal mucositis, in which one can readily see dental impressions, are somewhat more ominous than the lesser oral lesions.

Dysphagia, as a manifestation of esophageal ulceration and esophagitis, is a symptom of serious intoxication. Abdominal pain, diarrhea, perianal or perivulvar mucositis and dermatitis, and papulopustular folliculitis also occur at relatively moderate doses and, even though less commonly, as the first sign of intoxication. Anorexia is common with lesions of the alimentary canal. Sensitivity of the oral mucosa and unrecognized ulcers to acidic stimuli, such as citrus fruit juice, can be a valuable sign.

Table III. *Rates of response to treatment of acute myelocytic leukemia in patients more than 20 years of age*

Program	No. of patients	Complete remission (%)	Complete remission with corticosteroids (%)	Partial remission with or without corticosteroids (%)	Total remission status (%)
First					
Amethopterin	32	3		3	6
6-Mercaptopurine	13		8	8	16
Second					
6-Mercaptopurine	30	10		7	17
Amethopterin	8				0
Third					
Amethopterin plus 6-Mercaptopurine	34	9	6		15

Data from Acute Leukemia Study Group B, Clinical Studies Panel, CCNSC, E. Frei, III, Chairman: A study of sequential and combination chemotherapy with antimetabolites (Methotrexate and 6-mercaptopurine) in acute leukemia. Unpublished observations, 1960.

More vigorous drug administration leads to marrow suppression with leukopenia, reticulocytopenia, and thrombocytopenia. Less commonly, these effects are seen without any alimentary tract lesions. Toxic pancytopenia may be difficult to distinguish from subleukemic leukemia per se.

One or 2 days' observation of a possible toxic effect after discontinuing administration of the drug will ordinarily clarify the diagnosis. It is wise, upon reinstitution of amethopterin, to start at one-half dose for a week after a bout of intoxication has cleared. In the presence of substantial leukopenia, marrow aspiration is essential to determine if one is suppressing a regenerating hematopoietic tissue or a wholly leukemic marrow. Leukopenia in the presence of a cellular leukemic marrow is not a basis to stop administration of the drug.

Administration of citrovorum factor to patients receiving folic acid antagonists can lead to healing of toxic ulcerations despite continuation of the antagonist. The time relationships do not clearly establish that citrovorum factor accelerates healing. It seems more reasonable to believe that in the presence of large amounts of exogenous citrovorum factor, folic acid antagonists are ineffectual at standard doses and healing results when further folic acid antagonism is prevented.[144] Concomitant administration of citrovorum factor and amethopterin in acute leukemia allowed many times more amethopterin to be tolerated but did not enhance therapeutic efficacy.[145]

Other schedules for amethopterin administration to patients with leukemia have been presented. Much of the basis for these schedules derives from observations by Goldin and his co-workers[146] on the inferiority of other schedules to one giving 4-aminofolic acid every fourth day in leukemia L-1210 shortly after transplantation. Goldin and colleagues[147] have reported, however, that in mice with advanced leukemia, daily administration of amethopterin was superior to less frequent administration.

Three times the daily dose of amethop-

terin given every third day to 32 patients with acute leukemia was contrasted to the daily administration of the drug to 33 patients. Although the patients were randomly allocated to the two amethopterin regimens, all 65 also received 6-mercaptopurine daily at full dose level. No significant difference in remission rate or survival could be attributed to the schedule of amethopterin. Among those patients who did have a remission, however, the daily administration of amethopterin seemed to produce longer remissions.[148]

The effects of large infrequent doses of amethopterin in acute leukemia (1 to 5 mg. per kilogram every 2 to 4 weeks) have recently been the subject of preliminary reports.[149, 150] Three of 8 children attained some degree of remission status, but profound anorexia and other impressive toxicity without obvious important concomitant therapeutic advantage suggest that this regimen will prove too toxic for clinical use.

Attempts to define the biochemical characteristics of sensitivity and resistance to drugs in human leukemic cells are still at an early stage of development.[21, 63, 74] Recent observations have indicated that acute leukemic leukocytes contain approximately twice the level of enzymes involved in folic acid metabolism (such as serine hydroxymethylase, cyclohydrolase, and others) as do normal leukocytes. This enhanced enzymatic activity was present at the same time that lower levels of glucose-6-phosphate dehydrogenase were found, thus demonstrating a qualitative change in enzymatic pattern.[151]

Intrathecal administration of folic acid antagonists for central nervous system leukemia

The emergence of central nervous system leukemia as a common manifestation of chemotherapeutically modified acute leukemia promoted interest in the concentrations of folic acid antagonists reaching the cerebrospinal fluid and the feasibility of direct installation of drug into the sub-

arachnoid space. Using microbiologic assay, Whiteside and coauthors[152] found that after 0.33 mg. per kilogram of amethopterin (10 mg.) given orally to a boy with lymphosarcoma, amethopterin appeared in the cerebrospinal fluid at 1 hour, reached a maximum of 15 mγ per milliliter at 3 hours, and fell to 3 mγ per milliliter at 18 hours. Serum levels, however, reached a peak of 900 mγ per milliliter at ½ hour and were down to approximately 60 mγ per milliliter at 3 hours. By contrast, intrathecal administration of 0.1 mg. of amethopterin per kilogram in 4 patients resulted in cerebrospinal fluid levels of approximately 5,000 mγ per milliliter at 15 minutes, with simultaneous serum levels of 20 to 60 mγ per milliliter. At 48 hours, cerebrospinal fluid levels in five studies after a single intrathecal dose of 0.1 to 0.5 mg. of amethopterin per kilogram ranged from 20 to 500 mγ per milliliter, the concentration being approximately proportional to drug dose, whereas serum levels were 2 to 21 mγ per milliliter. Clearly then, higher cerebrospinal fluid concentrations of amethopterin could be established with subarachnoid instillation. Cramblett[153] found 20 to 60 mγ of amethopterin per milliliter in the cerebrospinal fluid on the third day after intrathecal administration.

Clinical recognition of "cerebral leukemia" was less common before the advent of chemotherapy. Only 1 pediatric case of acute leukemia with symptoms of central nervous system infiltration was found in a literature survey.[154] With the near universality of chemotherapy for leukemia, the syndrome has become much more prominent. In one large clinic, approximately 4 per cent of children have this complication by the time they have survived 3 months, and by the median survival time of 1 year, one-third of children have developed it.*[155]

Central nervous system leukemia frequently appears at a time when systemic leukemia is otherwise clinically undetectable or markedly suppressed during complete or partial remission. It is probable that sequestration of leukemic cells beyond the blood-brain barrier in the subarachnoid space or brain substance decreases their exposure to critically sufficient concentrations of antileukemic drugs, including folic acid antagonists. This mechanism has been considered as the basis for intracranial pathologic states. In addition, sequestered leukemic cells might serve as a nidus for repopulation of marrow and other systemic tissues, themselves successfully rid of leukemic infiltration because of higher concentrations of drug.[82]

Sansone[156] first reported the use of 4-amino folic acid intrathecally to penetrate, although mechanically, the blood-brain barrier. Sharp drop of leukocyte counts in the cerebrospinal fluid of 2 boys with signs of "cerebral leukemia" was accompanied by clinical improvement in 1, but the response was transient. Since that time, many patients have been treated with intrathecal amethopterin. Whiteside and his coworkers[152] obtained improvement in all of 5 children with central nervous system leukemia or lymphosarcoma. Symptoms began to abate in 4 to 7 days, with reduction or disappearance of papilledema in 1 to 2 weeks. Improvement lasted for 4 to 6 weeks.

Cramblett reported 4 children who sustained improvement of the symptoms and signs associated with central nervous system leukemia. His patient 2 is of note because intrathecal administration of 5 mg. of amethopterin on three occasions, each 5 days apart, produced complete symptomatic remission and return to normal spinal fluid findings, the last only 7 days before death. At autopsy, however, extensive infiltration of the dura and leptomeninges was found.[153]

The largest reported experience in one clinic with various treatment for central nervous system leukemia covers forty-eight episodes in 32 patients.[155] In 2 patients, the central nervous system disease was the

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*C. P. Hyman, C. A. Brubaker, E. Borda, C. D. Hammond, and P. Sturgeon: Personal communication, 1960.

presenting symptom of leukemia, and in the remaining 30, it appeared 40 to 928 days (median 289 days) after onset of the systemic evidence of leukemia. In 8 patients, the central nervous system abnormalities were the only evidence of active leukemia at the time; the marrow, blood, and other organs of these children were normal.*

Fortunately, some concept of the natural course of disease without amethopterin is provided from this study.[155] Symptoms in four of ten episodes of central nervous system leukemia in 8 patients subsided spontaneously. No lumbar puncture was performed in this group either for diagnosis or therapy. Five of another ten episodes in 8 patients subsided completely after lumbar puncture alone. In the two groups, some of the symptomatic remissions may have been related to the introduction of corticosteroids. Eight of eleven symptomatic episodes of central nervous system leukemia in 9 patients were relieved by radiotherapy for a median duration of 61 days.

In one child who developed symptoms of central nervous system involvement while receiving maintenance 4-aminofolic acid, oral drug was increased until she became severely intoxicated. Symptomatic neurologic remission ensued for 105 days. It seems reasonable to believe that higher concentrations of drug penetrated the blood-brain barrier.

Since November, 1957, Hyman and her associates have treated twenty-one episodes in 19 patients with 0.2 mg. of intrathecal amethopterin per kilogram given on alternate days for three to six doses, ordinarily a total of 0.8 mg. per kilogram in four doses. Symptomatic relief occurred in eighteen of the twenty-one episodes. In seven of these instances, however, symptomatic relief followed diagnostic lumbar puncture and preceded intrathecal amethopterin.

The median symptomatic improvement for the group in whom complete relief occurred lasted only 75 days. One refractory patient had ascending paralysis which was halted but not reversed. The other two refractory patients had systemic disease which had been resistant to folic acid antagonist therapy from the outset.*[155] Benefit from intrathecal amethopterin in central nervous system leukemia has been seen by many observers, however, in patients in whom systemic relapse from amethopterin-induced remissions has occurred. This suggests that the higher concentrations which can be reached within the confines of the subarachnoid space are enough to affect partially resistant cells or that sensitive members of the original leukemic population have survived in long-standing infiltrates of the central nervous system and its membranes.

In many, but not all, patients on whom data are available, certain abnormalities of the cerebrospinal fluid are repeatedly recognized in central nervous system leukemia. Increased pressure, pleocytosis with lymphocytes and blasts, hypoglycorrhachia, and increased protein concentration may be present in varying combinations and degrees. These findings may all revert to normal beginning about 1 week after the start of intrathecal amethopterin. Their return to normal, particularly the lowering of cell count, does not represent abolition of the leukemic infiltration of the meninges or brain substance, however. Persistence or recurrence of the infiltration is the rule rather than the exception. Thus, intrathecal amethopterin constitutes a useful palliative measure in the control of established leukemia of the central nervous system, a common manifestation of the chemotherapeutically modified disease. Its more important role, however, may possibly lie in helping to eradicate leukemic cells beyond the blood-brain barrier at the outset of antileukemic treatment in patients with no ap-

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*C. P. Hyman, C. A. Brubaker, E. Borda, C. D. Hammond, and P. Sturgeon: Personal communication, 1960.

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parent leukemia of the central nervous system. Appropriate clinical studies directed at this problem have begun.[*]

Other neoplastic disease

The folic acid antagonists have not found an accepted place in the treatment of other advanced cancers. In a large series treated with several different folic acid antagonists, 8 of 49 patients with carcinoma and 16 of 44 with sarcoma and leukemia were described as sustaining objective improvements.[157] Some of these improvements seem of little clinical use to us now, armed with 9 years' hindsight and several similar therapeutic experiences. The 93 patients sustained 116 bouts of drug intoxication, which reflects the very narrow differential selectivity of drug effect for the tumors studied. Other reports confirm the low degree of activity in miscellaneous advanced cancers.[158, 159]

Recently, 36 women with advanced breast cancer have been treated with amethopterin in daily oral doses of 2.5 to 5 mg., although occasionally a patient received as much as 12.5 mg. per day. Objective improvement was observed in 10 of the 36 patients and began 2 to 12 weeks after onset. The duration of objective improvement varied from 3 weeks to 17 months, with a median of approximately 8 weeks. Another 9 patients showed equivocal improvement; 17 patients failed to benefit.[160] These results were attained without extraordinary evidence of toxic effects and are somewhat better than those reported in earlier studies of folic acid antagonist treatment of breast cancer.[161]

On the basis of observations of Hertz[162] that folic acid antagonist treatment blocked estrogen-stimulated growth of the immature genital tract in several species, amethopterin has been used in an attempt to interfere with estrogen-induced (and androgen-induced) hypercalcemia in patients with metastatic breast cancer.[163] Hypercal-

cemia did not reappear if hormone was added after amethopterin administration had begun, but elevation in calcium concentration might again occur when amethopterin was stopped. This technique, which was effective at levels of mild leukopenia, may allow hormone-induced remissions of breast cancer in some patients who otherwise might have been prevented from reaching remission by hypercalcemia.

Parenteral administration of repeated large infrequent doses of amethopterin (2.5 to 10 mg. per kilogram) to 84 patients with a wide variety of types of advanced cancer produced objective evidence of tumor regression in 20. The duration of response varied from 2 to 9 weeks, with a median of 4 weeks. Substantial intoxication occurred in 29 of the 84 patients. It is of interest that three of eight carcinomas of the tongue and pharynx demonstrated objective decrease in tumor size.[*]

Sixteen patients with advanced cancer were treated[†] with the oral amethopterin regimen introduced by Li, Hertz, and Spencer[113] for choriocarcinoma. Inconsequential tumor responses were seen.

Some activity of folic acid antagonists has been seen in chronic myelocytic leukemia by several authors, but the consensus is that remissions are short and inferior to those obtained with many other drugs.[159, 164, 165]

A study of folic acid antagonists in lymphomas has recently been undertaken[‡] in an effort to see if chemotherapeutic data from L-1210 lymphocytic neoplasm of mice[166] could serve as a useful prediction index. One can say at this time that amethopterin and dichloramethopterin have recognizable antitumor activity in lymphosarcoma and reticulum cell sarcoma. Their therapeutic status has not yet been defined.

The use of amethopterin for multiple

[*]Acute Leukemia Study Group B. Clinical Studies Panel, CCNSC, E. Frei, III, Chairman: Unpublished data, 1961.

[*]B. Shnider, A. Owens, and P. Condit: Personal communication, 1960.

[†]W. Regelson and J. F. Holland: Unpublished observations.

[‡]Eastern Solid Tumor Study Group, CCNSC, C. G. Zubrod, Chairman: Unpublished data.

basal cell carcinomas (in some patients, numbering in the hundreds) has recently been studied by Van Scott.* Approximately 1 week after intravenous injection of a single large dose of amethopterin, a marked reaction occurred in the tumors, characterized by bleeding and purulent exudation. Absence of mitoses and cellular disintegration were seen histologically. Those basal cell carcinomas which were erythematous originally (perhaps because of faster growth or some partial host defense) were the most responsive to drug. After a variable period, most of the basal cell carcinomas recovered from the injury and resumed growth, although repeated doses of amethopterin again might be temporarily effective. A few lesions clinically disappeared.

The tumor response was accomplished at drug levels that did not induce clinical changes in the normal skin, although oral mucosal lesions were sometimes seen.

Regional administration of amethopterin

An application of metabolite counteraction of antimetabolite effect has recently been reported by Sullivan, Miller, and Sikes.[167] These investigators have attempted a pharmacologic division of the patient into two areas: (1) a tumor-bearing area undergoing drug treatment within a regional circulation and (2) the remainder of the body receiving metabolite as protection against drug effect. Amethopterin has been administered by constant intra-arterial infusion in the appropriate regional circulation to provide intense exposure of the neoplasm to drug, while high doses of citrovorum factor were administered intramuscularly to compete with the effect on sensitive structures such as intestinal mucosa and marrow. After percutaneous or operative insertion of a polyethylene catheter, angiography was performed to demonstrate the arterial bed to be infused. Thirteen men received arterial infusions

*E. J. Van Scott: Personal communication.

for inoperable epidermoid carcinomas arising from, or recurrent in, the oral, pharyngeal, nasal, or sinus mucosa. Four of the 13 obtained substantial decrease in tumor size with marked local improvement, and an additional 3 of the 13 had partial tumor regression. The survival times of 4 of these 7 responsive patients were 11, 24, 53, and 64 days, and 3 patients with major improvement were alive at 30+, 44+, and 150+ days. In 6 of the 7 patients, leukopenia (400 to 2,000 per cu. mm.) and thrombocytopenia (40,000 to 110,000 per cu. mm.) appeared, indicating that important systemic drug effects were occurring despite administration of citrovorum factor.

To assess the importance of these systemic effects of amethopterin, the drug was infused intravenously in doses comparable to those used in the intra-arterial technique to 5 men with similar epidermoid cancers of the head and neck. These patients also received citrovorum factor in the same fashion as in the intra-arterial study. In 2 patients, a comparable degree of hematologic depression was produced (leukocytes 500 and 1,900 per cu. mm., platelets 50,000 and 90,000 per cu. mm., respectively) as in the intra-arterial responders. These 2 men had partial regressions of tumor size for about 3 weeks each. One died on the thirty-fourth day. In the remaining 3 men, no tumor response was seen, although systemic effects of the drug were less prominent: leukocytes 4,200, 3,500, and presumably normal levels (in the last instance with moderate oral toxic lesions). Other patients with miscellaneous tumors were treated with amethopterin, 4 intraarterially and 4 intravenously. Two transient responses (reticulum cell sarcoma and chronic myelocytic leukemia) were both ascribable to systemic drug effect. Two deaths associated with drug intoxication occurred in these 8 patients with miscellaneous neoplasms.

Certainly, the aim of localized chemotherapy without prohibitive general toxicity is a valid approach to the regionalized incurable cancers often found in the

head and neck (and gynecologic) areas. Dose schedules that allow attainment of this goal have not yet been perfected. In their later cases, Sullivan and his colleagues decided on 50 mg. of amethopterin in a constant intra-arterial infusion daily for 6 to 10 days and on 6 to 9 mg. of citrovorum factor every 4 to 6 hours intramuscularly. The results in the intra-arterial and the intravenous infusion series do not establish beyond question that systemic drug effects did not play a major role in tumor regression. Further study of this regional system is merited and will doubtless define whether it offers therapeutic advantage not attainable in other ways.

Folic acid antagonists and psoriasis

The use of folic acid antagonists in rheumatoid arthritis and psoriasis was introduced by Gubner[168, 169] in 1951. Six of 7 patients with rheumatoid arthritis had transient increase in mobility and decrease in pain for periods lasting up to 6 weeks at dose levels of 4-aminofolic acid which caused intoxication. Neutropenia was not observed.

Psoriatic scaling stopped on the fifth to the tenth day in all 13 patients with this disease who were treated. Multiple small areas of hemorrhagic crusting appeared in psoriatic lesions, followed by separation of the crusts in plaquelike aggregates, leaving a residual purple epithelized area which faded. Relapse of skin lesions occurred 3 to 8 weeks after administration of the folic acid antagonist was stopped. One patient each with long-standing chronic atopic eczema, chronic eczematized seborrheic dermatitis, and chronic discoid lupus had marked improvement after single courses of 4-amino folic acid at toxic levels. In addition to oral ulceration in all patients, incomplete alopecia and diminished beard growth were seen in some. Delayed healing of a biopsy wound, proliferation of exuberant granulation tissue without apparent epithelial growth for 1 month, and excoriation of contacting skin surfaces, to-

gether with more impressive effects in psoriasis than in rheumatoid arthritis led to the postulate that 4 aminofolic acid exercises greater effect on epithelial than on connective tissues.

Widespread use of folic acid antagonists in the treatment of psoriasis has evolved. It is estimated that there are 4,000,000 psoriatic patients in the United States.[170] A recent report on 329 patients treated by one group with 4-aminofolic acid (0.5 mg. daily for 12 days) or, less commonly, with amethopterin emphasizes the extent of the therapeutic endeavor.[170]

Of the 329 patients, 31 had lasting benefit from a single course, a frequency accepted by many observers as compatible with the natural regression rate in psoriasis. In an earlier analysis of the first 171 patients of this group, relapse from whatever improvement had been achieved had occurred in 96 per cent by 5 months.[171] Maintenance treatment was instituted in many of these patients, thus providing the largest number of patients of any kind treated over long periods of time with folic acid antagonists. The incidence of recognized toxic effects in this large series was 28 per cent, although hematologic studies were admittedly incomplete. Forty-one of the 329 patients took more than sixteen courses of 4-aminofolic acid, each course consisting of 0.5 mg. daily for 12 days, usually every 21 to 28 days. The average total dose was 197 mg. taken in a period of 1 to 5 years. Forty of the 41 patients had improvement of 50 per cent or more in skin lesions.

Of the 329 patients, 82 per cent sustained 50 per cent or more improvement at some time. Treatment was discontinued in many for unspecified reasons. In 19 patients, however, resistance apparently developed. All had had more than 50 per cent clearing of the lesions originally, and 14 of the 19 had had more than 75 per cent regression. Seven of the 19 patients who failed to respond to a second course of 0.5 mg. daily for 12 days did subsequently respond to a more vigorous course. Twelve patients were

refractory to two or three additional courses, however, even at high dose levels. This is of interest as an example of acquired resistance in a rapidly growing epidermal hyperplastic tissue that is not a neoplasm.

The mechanism of action of amethopterin and other chemotherapeutic compounds in psoriasis has been studied by Van Scott.[71] His studies indicate that the mitotic arrest and inhibition of epidermal hyperplasia which occur after local application of colchicine or podophyllotoxin are not produced by systemic amethopterin. Rather, the effect of amethopterin is similar to that of local mercury and liquor carbonis detergens, with which inhibition of cell growth is apparently achieved by a different mechanism. One day after intravenous administration of amethopterin, no mitoses at all were seen in the skin. Interruption of the hyperplastic overgrowth was accompanied by increased formation of cytoplasmic keratinous processes (tonofibrils) and keratinization.

It is of interest that all three dermatologic investigators cited above were unable to produce regression of psoriatic lesions by local application of 1 per cent 4-aminofolic acid ointment to 4 patients,[169] 1 per cent 4-aminofolic acid ointment to 12 patients[171] or amethopterin as a powder or in acid or alkaline solution, even after removal of the stratum corneum by cellulose tape.[71] In the latter instance, some transdermal absorption was proved by the finding of amethopterin in the urine. These findings were interpreted as evidence that the pharmacologic activity of amethopterin might be initiated at a distant site.[71]

It is fortunate that the experience in psoriasis is already gained and behind us. One cannot but hope that the toxicity and potential hazards, when added to the 96 per cent relapse rate within 5 months after receiving drug,[171] will discourage the use of folic acid antagonists in ambulatory psoriatic patients. In those patients in whom the total syndrome of disease from psoriasis (with or without arthritis) is suf-

ficiently incapacitating, life threatening, or refractory to the many other less dangerous and often helpful therapies to merit the risk, the folic acid antagonists may provide temporary benefit. However, the long-term effects of folic acid antagonists, on marrow function, including leukemogenesis, on the alimentary canal, or hepatic function, and on the integrity of the dorsolateral columns of the spinal cord are unknown quantities.

Folic acid antagonists, viruses, and immunity

A relationship between the manifestations of viral infection and folic acid metabolism was early noted in the course of investigating the properties of folic acid antagonists. Rous sarcoma was completely inhibited for at least 16 days after implantation into newly hatched chicks fed on a folic acid deficient diet. Addition of 100 γ of folic acid per chick per day allowed 90 per cent of the tumors to grow. Comparative ranking of folic acid antagonists in treating this tumor in chicks fed on regular mash disclosed that both amethopterin and 4-aminofolic acid were active, the latter being the most effective folic acid antagonist tried.[172]

Amethopterin was found to have no therapeutic effect for mice infected with laboratory-adapted strains of feline pneumonitis, vaccinia, influenza, western equine encephalomyelitis, or poliomyelitis.[173] None of sixty various strains of bacterial viruses were completely inhibited by amethopterin, although ten were partially suppressed.[174]

Recent chemotherapeutic trial of amethopterin against the Friend virus leukemia of mice revealed 54 per cent inhibition of splenic enlargement when measured at 3 weeks. The significance of this result is tempered by the fact that forty-seven compounds of the 110 tested had greater activity.[175]

Evidence has accumulated that the effects of folic acid antagonists in some virus-cell-host interactions are not dependent on interference with virus proliferation. Haas

and his co-workers have shown that mice inoculated with ordinarily lethal doses of lymphocytic choriomeningitis virus can survive if treated with amethopterin,[176] or with dietary folic acid deficiency.[177] The sparing effects of amethopterin or folic acid deficiency could be dissipated by administration of citrovorum factor. Viremia is prolonged in amethopterin-treated mice. When nonlethal, low doses of lymphocytic choriomeningitis virus were given to control mice, no viremia could be demonstrated and immunity rapidly developed. In a group treated with amethopterin, however, viremia was demonstrable by cardiac blood passage for more than 4 weeks.[178] Titrations of intracerebral virus in amethopterin-treated survivors showed concentrations similar to those of control animals which died. It was concluded that survival from the effects of folic acid deficiency or antagonism is not due to a difference in virus production. Furthermore, since the disease produced in test animals in the course of determining virus titers was entirely typical of acute lymphocytic choriomeningitis, the increased survival in the amethopterin-treated animals was not attributable to reduced virulence of the virus.[179]

Although the pathologic findings in twenty-two animals examined early after amethopterin treatment suggested suppression of inflammatory response in all but four, studies on twelve animals killed from the twenty-first to fifty-sixth day revealed eight instances of full-blown lymphocytic choriomeningitis. Amethopterin did not eliminate but rather delayed the inflammatory response. The presence of virus and of cellular infiltration without causing death suggests that ordinarily death from lymphocytic choriomengitis is not attributable to these two phenomena alone.[179] A direct implication of folic acid metabolism in a viral disease is thus apparent. Humoral factors are being studied.

Several lymphocytic neoplasms carry lymphocytic choriomeningitis virus as a contaminant.[180] An amethopterin-resistant tumor, P-288/A, was deliberately contaminated with lymphocytic choriomeningitis virus during in vitro incubation. The tumor was then transplanted to mice immune to lymphocytic choriomeningitis. No viremia was detectable. In amethopterin-treated immune animals, however, sufficient compromise of host defense was produced to allow for viremia.[181]

Compromise of human immunity to viral infection has been suspected in children with acute leukemia or other neoplasms treated with amethopterin. Fatal varicella developed in a child with neuroblastoma who was taking amethopterin.[182] A fatal instance of giant cell pneumonia with a terminal measles exanthem has been described in an infant with acute leukemia in complete remission during amethopterin treatment.[183] Recently, 4 other children with acute leukemia in complete remission from amethopterin administration have developed severe, prolonged, or fatal measles, confirmed by recovery of the virus.[184] Persistence of the virus and depressed antibody formation to it were observed. No severe measles of this type was seen in comtemporary cases in the area, neither had prior measles in patients with acute leukemia been recognized to run this course.[184] It seems reasonable to ascribe a role to amethopterin in this interaction between virus and leukemic host. It is of note that this did not result in the symbiosis seen in mice with lymphocytic choriomeningitis infection treated with amethopterin.

The effect of amethopterin on immune response has been demonstrated in other systems involving cellular antigens. Five of eight strains of mice with genetic histologic incompatability for proliferation of the amethopterin-resistant tumor P-288/A allowed progressive growth of the neoplasm when amethopterin treatment was given.[181] This drug-permitted proliferation was interpreted as evidence that an interference with host cell response to the homografted tumor had occurred.

In experiments with homologous marrow transplantation from a parent strain to f_1 hybrid mice irradiated to the LD_{98} dose,

only one of six mice survived.[185] When the irradiated mice with transplants were treated with amethopterin starting 14 days after the graft, however, four of seven mice survived 70 days and looked as well as mice who had received an isologous marrow transplant. The three of seven who died apparently succumbed to a cause other than homograft reaction. In this experimental system, it was necessary to give drug at toxic levels in order to avoid delayed homograft reaction. In another marrow donor-recipient pair, no improvement in survival occurred. Favorable influence on skin graft survival was seen in some instances.[185] It may be that timing, toxicity, and host specificity will prove too nettling to allow translation of these observations to wider usefulness. The heuristic value of such observations, however, and the possibilities of modifying cellular and/or humoral immune responses merit high priority for investigation.

Experiments to detect an effect of amethopterin on antibody formation to a protein antigen in rabbits, in a system where 6-mercaptopurine is highly effective, have thus far been negative, although the maximum tolerated dose of the drug has not yet been reached.*[186]

An effect of 4-aminofolic acid on leukemogenesis has been reported in a small group of rats. Two generations of albino rats received 4-aminofolic acid in their food continuously. The third, fourth, and fifth generations were then studied. Four of nine rats in the fourth and fifth generations developed subacute myelocytic leukemia. Three of twelve rats in the second and third generation had hematologic and morphologic changes compatible with preleukemia. Four generations of control rats were free from blood changes.[187] Extension of these studies might provide a rich yield.

New antagonists

Passing reference has been made to evaluation of dichloramethopterin in the treatment of choriocarcinoma and lymphoma. Although very many structural analogues of folic acid and the pteridine nucleus and several 2,4-diaminopyrimidines have been studied for antifolic activity, there would appear to be no recognized major advantage to any of these drugs in clinical medicine. A major exception is seen in the unique activities of pyrimethamine, considered previously in this symposium.[190]

In mice bearing leukemia L-1210, the substantial increase of antileukemic activity shown by 3′,5′-dichloramethopterin, 3′-chloro-5′-bromamethopterin, and to a lesser extent by other monohalogenated derivatives is noteworthy. The survival time of mice with advanced leukemia at onset of treatment has been extended from 2 or 3 days untreated and 28 days when treated with amethopterin to over 100 days when optimal doses of the new antagonists were given. Some mice were presumably cured of the disease.[166]

Recognition of enhanced activity of 3′,5′-dichloramethopterin only when given parenterally to mice with leukemia[188] is serving as the basis for clinical trial at present.

It seems an admissible presumption, regardless of the outcome of the clinical trial with these particular drugs, that better folic acid antagonists may yet be discovered and gain a more extensive place in the therapy of human cancer.

In addition, the effects of dietary deficiency of folic acid in the rat have recently been shown to inhibit growth of Walker carcinosarcoma 256, whereas amethopterin did not do so.[189]

This effectiveness of vitamin deficiency indicates pathways dependent on folic acid metabolism but uninhibited by amethopterin. It is possible that similar effectiveness in inhibiting neoplastic growth may be found in other animal tumors and in man.

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*R. Schwartz: Personal communication, 1960.

Addendum

Since completion of this review in June, 1960, several reports have appeared which merit at least brief mention.

The demonstration of dihydrofolic reductase in the leukocytes of all of 12 patients with acute leukemia and in chronic myelocytic leukemia, but not in normal leukocytes, chronic lymphocytic leukemic leukocytes, or erythrocytes, is of importance. The human enzyme was inhibited at the same low drug levels as the enzyme from other sources, leading to the surmise that it was indeed a primary target for folic acid antagonist therapy.[191] In 6 patients with acute leukemia treated with amethopterin, dihydrofolic reductase increased as much as five to twenty fold in association with the development of resistance.[192, 193] This increased enzyme might tightly bind whatever drug gained entry to the cell, and the susceptibility of the patient's normal cells precludes higher dose. Leukocytes and erythrocytes from patients with nonhematopoietic neoplasms contain appreciable levels of folic reductase after amethopterin treatment, too, indicating that cellular adaptation in these normal cells also can occur.[193]

Results of some investigations with dichloramethopterin have appeared. A comparative study against amethopterin, when both drugs were given orally, revealed no difference in 30 patients less than 20 years old with acute leukemia. Subsequent discovery that the advantage of dichloramethopterin in mice obtained only after parenteral administration[188] led to adequate parenteral trial in another 30 individuals less than 20 years old with acute leukemia. The complete remission rate was only seven per cent.[194] A partial explanation for the failure of the superiority of dichloramethopterin in mice to be observed in man lies in differential rates of human metabolism of the two drugs. Using the best technique available for measuring 4-aminopteroylglutamic acid compounds—the binding to folic acid reductase[33]—it was found that by 4.5 hours, the serum levels of dichloramethop-

terin and amethopterin were equal, despite the fact that five times as much of the dichlorinated compound had been given. Since the same proportion of each compound was excreted in the urine, the metabolism of dichloramethopterin to an inactive compound must be much more rapid than that of amethopterin.[195]

The disposition and metabolism of Cl^{36}-labeled dichloramethopterin has also been reported. About 50 to 65 per cent of parenterally administered drug appears rapidly in the bile, of which the majority in man occurs as the 4,7-dihydroxy derivative of dichloramethopterin and the remainder as the unmetabolized drug. Recovery in urine and feces of drug and the single metabolite was 90 to 95 per cent in 24 hours.[196]

Continuing experience with the arterial infusion of amethopterin with intramuscular citrovorum factor administration has been favorably reported.[197] Significant objective evidence of tumor regression within the infused arterial bed has been described in 27 of 38 patients with epidermoid carcinoma of the head and neck and in 5 of 9 patients with carcinoma of the cervix. Eight persons were observed to sustain complete tumor regression, although some such patients have subsequently had relapses.

Amethopterin suppressed skin hypersensitivity and antibody production following immunization with diphtheria toxoid or ovalbumin in guinea pigs. The tuberculin reaction was also suppressed at higher drug dose.[198]

The isolation from horse liver of an essentially pure form of a folic acid compound, prefolic acid A, which has spectral similarities to tetrahydrofolic acid, has been reported. The compound can be oxidized to tetrahydrofolic acid, and tetrahydrofolic acid can be reduced to prefolic acid A.[199] Its chemical identity has not yet been established.

Larrabee and Buchanan have indicated the existence of a new tetrahydrofolate cofactor containing a one carbon substituent. With a suitable *E. coli* enzyme and other cofactors, the one carbon fragment is do-

nated to homocysteine to form methionine, and tetrahydrofolate is released. The new cofactor does not replace folate or tetrahydrofolate as a growth factor for *Str. faecalis* or *L. citrovorum*. Preliminary indications are that the one carbon fragment is a methyl group.[200]

Wahbe and Friedkin[201] have presented persuasive evidence that dihydrofolate is formed in equimolar proportion from 5,10-methylenetetrahydrofolate when deoxyuridylate is converted to thymidylate. Thus, the donation of tetrahydrofolate hydrogen to the methyl group of thymidylate, leaving dihydrofolate, appears established.

Other investigations on the biosynthesis of thymidylate with extracts of *Str. faecalis R* have suggested that 4-aminofolic acid is without effect if substrate amounts of tetrahydrofolic acid are present. If only catalytic amounts of tetrahydrofolate or dihydrofolate were present, however, the necessary reduction to regenerate tetrahydrofolate was markedly sensitive to 4-aminofolic acid. In this system, DPNH appeared to be a better reductant of dihydrofolate than TPNH.[202]

The recent synthesis of tetrahydroaminofolic acid under conditions designed to protect it from oxidative destruction has been reported. Whereas early reports of decreased activity of presumably unprotected hydrogenation products of 4-aminofolic acid have been published, the tetrahydroaminofolic acid prepared by Kisliuk showed considerable enhancement of antifolic activity on *Str. faecalis* and on *Ped. cerevisiae*. The inhibition was readily reversed by citrovorum factor, however, suggesting that tetrahydroaminofolic acid does not bind tightly to its target enzyme sites, in contrast to the nearly irreversible binding to folic reductase of the unreduced homologue, 4-aminofolic acid.[203]

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(To be concluded)

Book reviews

Disease and the Advancement of Basic Science, edited by H. K. Beecher. Cambridge, Mass., 1960, Harvard University Press. 416 pages. $12.50.

The Lowell lectures of 1958, edited by Professor Beecher and recorded in this volume, are intended to corroborate the thesis that the study of disease processes in man constitutes an important, and in some cases unique, aspect of the pursuit of fundamental natural science. The validity of this general thesis is most convincingly illustrated by the contributions of twenty-one authors, each a leading authority in his speciality.

The choice and scope of the diverse topics attribute an almost encyclopedic character to this volume, with a strong emphasis on tracing the development of fruitful current ideas and concepts in medical sciences to their origin. Beyond recording authoritatively these accounts in which all contributors, in their respective fields, played an eminent role, it seems to me that the entire monograph is pervaded by a profound and scholarly sense of historic continuity in science, by respect for the creative investigator, and by a deep appreciation of the social responsibility of medi-

cal science. The latter is most strongly emphasized by René J. Dubos, who delineates aspects of medicine which fall into the realm of "social technology" and who explores effects of the physician's activity on the ecology of the species of man.

The most obvious way in which basic medical science can benefit from the study of disease processes in man is undoubtedly provided by those instances in which disease—in Professor Beecher's physicalistic words—impresses a perturbation on the organism. In this, disease resembles the experiment, except that it occurs without deliberate planning. Traumatic brain lesions, endocrine disorders, and disturbances of nutrition, intermediary metabolism, and homeostatic forces attest to the significance of this view and are competently illustrated by the lecturers on these topics. Occurrences of these disorders are instrumental in delineating the "normal"; and if explanation of natural phenomena, as is generally believed, consists of imposing order and context to individual occurrences, the abnormal should be as much encompassed by the explanation as is the normal.

There are also other, more unpredictable and subtle ways in which clinical observation can assume significance for funda-

mental biologic comprehension. P. Weiss emphasizes the "unbroken continuum of life processes" and, no matter at what level of complexity one enters the exploration of life processes, there is no way of telling what will prompt an illuminating idea or an original flash of insight—"a clinical observation or a laboratory experiment on amphibian embryos." Professor Beecher's study of subjective responses exemplifies yet another role of clinical research: quantitation of sensation and mood is capable of revealing the personal and private aspect of subject-environment interactions and its modification by drugs. Thereby, clinical research adds fundamental insight into human behavior and prevents knowledge of drug effects from being deprived of a singularly important dimension.

This inspiring volume, apart from presenting a delightful collection of illuminating lectures on a large variety of topics in academic and practical medicine, convincingly attests to two issues: that natural science cannot flourish, neither indeed can it exist at all, *in vacuo* and that there is a basis unity of medical sciences. In that sense, this lecture series implements most fittingly Sir William Bragg's view that "there is only one natural world, and only one knowlede of it."

Gerhard Werner

The Metabolism of Cardiac Glycosides, by S. E. Wright. Springfield, Ill., 1960, Charles C Thomas, Publisher. 86 pages. $4.75.

In a matter of 74 pages, plus 12 devoted to a space-filling bibliography of 164 references, Dr. Wright presents a concise account of the cardiac glycosides. Although the title indicates that metabolism of the cardiac glycosides will be the major consideration, this turns out not to be the case. There are brief sections on chemical and structural considerations (7 pages); on an attempt, which is quite unsuccessful, to provide a basis for structure-activity relationships (9 pages); on methods for examining tissues and urine for glycosides (13 pages); on the absorption of the cardiac glycosides (5 pages); on their distribution in organs and tissues (11 pages); and on their excretion in the urine, bile, and intestine (10 pages). There remain 7 pages which are devoted to the metabolites of the glycosides that are thought to exist and to what little is known of them and 5 pages which cover in vitro investigations of metabolic problems. All is summed up in 5 pages. Total—86 pages.

The language, it is true, is simple and direct, but all in all, this small work adds up, at best, to a simple summary review of a few pharmacologic aspects of the cardiac glycosides; it fails to live up to the promise made in the title, *The Metabolism of Cardiac Glycosides.*

Walter Modell

Style Guide for Chemists, by L. F. Fieser and M. Fieser. New York, 1960, Reinhold Publishing Corporation. 116 pages. $2.95.

In addition to their important and authoritative books on organic chemistry, the Fiesers have written a small book on writing which for clarity, brevity, and directness is a wonderful example of what it recommends for others and, indeed, what they should attempt to emulate. The title unfortunately suggests that the book is for chemists only; actually the first 50 pages are applicable to all who would write. The remaining pages have information useful only for the chemist, but this should not deter the physician who writes from reading the first part of the book, which is one of the concisest statements on style, surpassed only, perhaps, by the White-Strunk *Elements of Style.* And if the physician already has that current best seller, I can see no need to have this book also.

Walter Modell

Toxic Phosphorus Esters: Chemistry, Metabolism, and Biological Effects, by Richard D. O'Brien. New York, 1960, Academic Press, Inc. 434 pages. $14.50.

This book is addressed to chemists and biologists and is intended to be a source book on organophosphate research and to show how our understanding of the events that follow organophosphate poisoning of animals can be explained in terms of events at the molecular level. Critical review of a large body of data and careful organization have enabled the author to accomplish this objective admirably.

The orientation is largely chemical. The reactions of the organophosphates in vitro are reviewed with emphasis on presentation of material which is of aid in understanding the in vivo reactions. The discussion generally utilizes the reactions of prototype compounds, but details of the reactions of many specific compounds are also included, usually in tabular form. Discussions of the nonenzymatic reactions, the effects of the compounds on cholinesterases, the effects of cholinesterases on the compounds, and synthetic and analytic techniques are included.

This material forms the basis for the discussion of the biologic actions of the organophosphates. The principal emphasis is on toxicity, both mammalian and insect, but considerable attention is given to other actions such as the effects on ion permeability and the metabolism of specific compounds. The section on nerve and muscle function as affected by the anticholinesterases is the clearest and most objective attempt to separate fact from theory in this field that I have seen. Particularly interesting is the section on selective toxicity where an attempt is made to correlate the structural and chemical properties of the various compounds with their relative toxicities for different species.

The organization of the book is excellent, the presentation clear and readable. An extensive bibliography is included in each chapter. The specialist is likely to find the coverage of his field too brief; he will find the review of related material informative and valuable. The neophyte will appreciate the skillful presentation of basic material and the careful integration of chemical and biologic data.

Frank G. Standaert

Handbook of Chemistry and Physics, forty-second edition, edited by C. D. Hodgman. Cleveland, 1960, Chemical Rubber Publishing Company. 3481 pages. $12.00.

As far back as I can remember, the *Handbook of Chemistry and Physics* has been a unique treasure chest of information. It has always seemed to contain all conceivable factual information on the many aspects of the broad field it covers, mainly in tabular form. And each (nearly annual) edition brought it completely up to date.

The book has grown enormously with the years, more than 1,000 pages in fifteen editions, to the present 3,500 closely packed pages. As new tables are added with each edition (twenty-two in the current one) and as those already included are expanded, it seems almost inevitable that despite the use of thinner stock, it will not be long before the book will have to be published in more than one volume. The current edition is 2½ inches thick and somewhat difficult to handle. As a matter of fact, the extensive mathematical tables and the tables for identification of organic compounds (expanded) are each available as separate volumes.

The forty-second edition, as many past, is divided into five sections: the mathematical tables, tables of properties and physical constants, general chemical tables, tables on heat and hygrometry, and tables on quantities and units. Each is a monumental collection of data.

In addition, there is a small section hidden among some more sophisticated mate-

rial which I recall as being very much the same 25 years ago; it is entitled "Laboratory Arts and Recipes" and explains such things as how to blue steel and iron, how to make De Khotinsky cement, how to make mirrors, and how to mount cross hairs for an optical system with fibers from spider webs. Although these may be useful, it seems unlikely that anyone would think of looking for them in this book. It is only fair to say also that, the *Handbook* notwithstanding, it is indeed very difficult to obtain spider web fibers from dealers in scientific apparatus. I would recommend this section be deleted except that I do not know where else one might find instructions on mounting spider web fibers. And it is a very short section and amusingly old fashioned.

The book is exceedingly well organized, the tables are easy to read, and, once one gets acquainted with the book, there is no trouble finding things. The editors are to be congratulated on maintaining the high standards of this book through the past 45 years. I think that every laboratory worker should have a copy of his own.

Walter Modell

New and Nonofficial Drugs 1961, issued under the direction and supervision of the Council on Drugs of the American Medical Association, Philadelphia, 1961, J. B. Lippincott Company. 849 pages. $4.00.

Much remains before this volume will attain the stature of its predecessor, *New and Nonofficial Remedies.* However, as an annual selection of proprietary drugs with detailed descriptions, it is still far superior to all others available, since, in varying degree, the latter tend to be merely uncritical collections of brochure literature. For better or worse, therefore, *New and Nonofficial Drugs 1961* is recommended.

Walter Modell

Physicians' Desk Reference 1961, fifteenth edition. Oradell, N. J., 1961, Medical Economics, Inc. 940 pages.

Modern Drug Encyclopedia, eighth edition; edited by H. D. Fein. New York, 1961, The Reuben H. Donnelley Corporation. 1649 pages. $17.50.

These books may well be reviewed together despite the fact that the first is given free to almost every practicing physician in the country and the other costs $17.50.

Both are listings of drugs currently on the market in this country. The arrangement of the descriptions is according to proprietary name; thus, the same drug may be described six or more times without any indication of this duplication. The descriptive material is patently supplied by the manufacturer.

Both books contain extensive manufacturer and therapeutic indices. Since the therapeutic indications seem to come from package material and manufacturer's brochures, the applicability of the drugs available seems vast and the number of drugs available for all of man's ills seems more than adequate.

The essential differences between the books, other than the cost, are that whereas the PDR is 1¼ inches thick, is printed on rather poor stock, and in general is a fairly skimpy job in which drug product descriptions are grouped according to manufacturer, the *Modern Drug Encyclopedia* is 2¼ inches thick, is well printed on good stock, has far more extensive quotations from package materials, and lists drug product descriptions alphabetically according to proprietary names. It is supplemented each month by a small addendum to keep it up to date. The *Modern Drug Encyclopedia* is published every 3 years and is well enough bound to stand up for longer than that. The monthly supplements are included in the price.

Neither book had an editorial policy that is apparent to me.

Walter Modell

American Drug Index 1961, by C. O. Wilson and T. E. Jones. Philadelphia, 1961, J. B. Lippincott Company. 791 pages. $6.75.

The *American Drug Index* represents an annual contribution by Doctors Wilson and Jones of considerable substance. It is the most comprehensive, compact listing of drugs and drug mixtures on the American drug market. Unlike some of the others, e.g., the *Modern Drug Encyclopedia* and the *Physicians' Desk Reference*, it lists drugs under generic as well as proprietary names and attempts to show which drugs with different trade names are the same materials. Thus one finds that methamphetamine is sold under more than twenty different trade names. It also indicates that methamphetamine is sold under various names in combination with aminophyllin, atropine, butabarbital, caffeine, chlor-trimeton, dehydrocodeinone, dehydrocholic acid, estrogens, ferrous gluconate, gramacidin, hyoscine, methapyrilene, methylcullulose, methyltestosterone, reserpine, salicylamide, sulfacetamide, sulfathiazole, thyroid, thyrotricin, and an assortment of vitamins. Little else is said about drugs besides their composition.

Keeping up with new drugs is, in itself, a stupendous undertaking, but the matter of the irrational drug combinations seems insuperable. Yet Doctors Wilson and Jones seem to have done it. I find the book most useful.

Walter Modell

Books received

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Conn, H. F., Editor: Current Therapy—1961, Philadelphia, 1961, W. B. Saunders Company. 806 pages. $12.50.

Faulconer, A., Jr., and Bickford, R. G.: Electroencephalography in Anesthesiology, Springfield, Ill., 1960, Charles C Thomas, Publisher. 90 pages. $4.75.

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Wilson, C. O., and Jones, T. E.: American Drug Index 1961, Philadelphia, 1961, J. B. Lippincott Company. 791 pages. $6.75.

Correspondence

The drug explosion

From a medical student

Modern day advertising, in my opinion, is an insult to one's intelligence. It is clearly designed to appeal to one's emotions with its glib claims of "biggest, best, fastest, safest, and most fashionable," rather than to one's sense of reason and good judgment. Obviously prudent business concerns would not continue this practice unless it gave the best results, so I take it as an indication of the level of intelligence of the average American.

It distresses me to see identically the same tactics being used by the pharmaceutical companies—the same subterfuge and falsehoods. Not that medical men aren't objective enough to avoid being taken in, but they must spend much valuable time analyzing the new drugs and claims by their producers, or they must resign themselves to missing the occasional new and more useful drugs that are produced. This task is great, even for a physician with much wisdom and experience.

But consider a student or young doctor. We are tender bait. We are besieged by free literature, free samples, complimentary reference books, journal advertisements, and the like in a mad rush by the pharmaceutical companies each trying to make his own impression while the "putty is soft." Traditional, proved pharmacology is obscured. Our pharmacology course seems like old-fashioned medicine. We lose sight of the fact that drugs are potent molecules acting as such inside a complex system of molecules upon which a life depends. Drugs are positively stated to be what they are not, and one drug might have as many as five or ten different names from as many producers.

For a young doctor, keenly aware of his responsibility for human life, devoted to his desire to provide the best care he can, and burdened with the task of learning a portion of medical knowledge, this exploitation by the pharmaceutical companies is confusing and misleading. I personally feel it is completely unjustified and immoral.

J. R. McTammany
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Durham, N. C.

From a practitioner

Obviously, from the editorial at the front of the book and Dr. Friend's proposals for more rational therapy at the end, you are greatly concerned about the current situa-

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tion with regard to the overwhelming flood of proprietary drugs. Perhaps it did not occur to you, but your lone entry in the Correspondence section contains the solution to so much of today's iatrogenic disease: "The reason . . . is from . . . the leech. [Remove the leech, and]—the sick person only after one week . . . becomes well!"

H. Clark Balmer, M.D.
266 Runnymede Rd.
Toronto, Ont.

From a teacher of clinical pharmacology

After reading your editorial in the current issue of CLINICAL PHARMACOLOGY AND THERAPEUTICS, I feel I must tell you how much I agree with all you have said and to let you know how much you have spoken for most of us in the field.

Aside from the harm and confusion done by the present state of affairs to patients and physicians, life for us in clinical pharmacology is being made steadily more difficult and our attention directed from the essentials to the multitude of the unessential and unnecessary, which none the less intrudes on medical practice.

I agree with you that self-control and self-education would be preferable to control from above, and I only hope that these sentiments will make headway in the right quarters.

Frederick Wolff, M.D.
Assistant Professor of Medicine
Division of Clinical Pharmacology
The Johns Hopkins Hospital
Baltimore, Md.

From a general pharmacist

Many pharmacists are just as upset as you concerning the vast duplication and "explosion" of "new" drugs in the past few years. I can look around the prescription department and see many, many examples of needless duplications in all sorts of types of drugs. Is it really necessary to have to carry ten different oral diuretics in stock? This does not even include the "logical combinations" of diuretics with other drugs. Is it really necessary for six different companies to market identical products of potassium phenethicillin? I shudder when I think of how many kinds of antihistamines must be on my shelves!

This duplication of similar products is one of the biggest headaches to the retail pharmacist and a great factor in the cost of prescriptions today. I hope that the pharmaceutical companies realize that the day of reckoning is here and that they will stop this outpouring of duplications and concentrate on developing really new compounds.

John Blower
Donnelly Pharmacy
1636 South Seventh St.
Terre Haute, Ind.

From a hospital pharmacist

Congratulations on the timely editorial, "The Drug Explosion," published in CLINICAL PHARMACOLOGY AND THERAPEUTICS. The editorial should penetrate the core of all discerning and discriminating prescribers who can indeed help stem the tide of "the drug explosion."

I would appreciate receiving twelve reprints of this editorial, if possible, in order that I may distribute it to key medical staff members of our hospital.

Claude U. Paoloni, Chief
Pharmacy-Central Supply
The Moses H. Cone Memorial
Hospital
Greensboro, N. C.

From the American College of Apothecaries

I would very much appreciate receiving 2,100 copies of your recent editorial, "The Drug Explosion," which appeared in a current issue of CLINICAL PHARMACOLOGY AND

THERAPEUTICS. We would like to distribute copies of this editorial to the Fellows of the American College of Apothecaries.

Robert E. Abrams
Executive Secretary
American College of Apothecaries
Hamilton Court
39th and Chestnut Sts.
Philadelphia, Pa.

From a drug consultant

I greatly enjoyed reading your editorial, "The Drug Explosion."

As far as Dr. D. P. Barr's statement that there are 140,000 medicaments in current use is concerned, this is the situation. At the time Dr. Barr published his statement, I checked into the matter and I learned that he had used as the basis of information a voluminous price list book known as *Drug Topics Red Book* and published by the Topics Publishing Co., Inc. This book publishes prices not merely on ethical drugs but on proprietaries, toilet goods and cosmetics, and other household merchandise. The figure of 140,000 does not even refer to the number of products, but to the number of items listed. That is, each new dosage form and each new package size of a product is considered a new item.

The total number of medicinal products is probably not larger than 12,000, and many of these are not marketed nationally.

Paul de Haen
11 West 42nd St.
New York, N. Y.

From a pharmaceutical manufacturer

As a scientist and a subscriber to CLINICAL PHARMACOLOGY AND THERAPEUTICS and more particularly as head of the research operations at The Upjohn Company, the editorial, "The Drug Explosion," in the January-February issue is of particular concern to me. . . .

If we are to address ourselves seriously to problems of this type, we must first be very certain that we are using the same guidelines and definitions. As a start in this direction, I would like to request that you supply me with answers to the following two apparently simple questions which I believe are crucial to the problems involved and on which there must be reasonable agreement:

1. List all of the "truly new drugs" that were made available during the 10 year period 1951 to 1960. Such a list would give us a fairly clear idea of what is meant by a "truly new drug."

2. What do you consider a reasonable cross-licensing arrangement? I think you will agree that delineation of the basic elements of such an arrangement are essential before trying to solve the problem.

R. S. Schreiber
Vice President for Scientific Administration
The Upjohn Company
Kalamazoo, Mich.

From a public welfare worker

As part-time Supervisor of Medical Services of the Essex County Department of Public Welfare, I have been trying for years to persuade doctors not to prescribe drugs just because they are new and frantically advertised, because of their expense and uselessness. (I could not stress the danger because it was not my function.) The reaction of Essex County doctors was violent. They would brook no interference or advice—except from detail men. . . . Your article is better than anything I have seen. In fact . . . I would like to send one to each physician in the county.

Harold J. Harris, M.D., F.A.C.P.
Westport, Essex County, N. Y.

Announcement

American Therapeutic Society

62nd Annual Meeting, Essex House, New York, N. Y., June 23-24, 1961

Scientific sessions

Friday, June 23

9:00 A.M. Opening Remarks
Arthur C. DeGraff, M.D.

9:05 A.M. Words of Welcome
William B. Rawls, M.D.

9:10 A.M. Presidential Address

President Harry E. Ungerleider, M.D., Presiding

9:20 A.M. The Bedside Challenge
Austin Smith, M.D., Washington, D. C.

9:35 A.M. Cardiac Work Evaluation: A Continuation of Cardiac Therapy
Arthur C. Kerkof, M.D., Henry W. Blackburn, M.D. (by invitation), and Charles P. Kolars, M.D. (by invitation), Minneapolis

9:50 A.M. Evaluation of Coronary Artery Vasodilators
Arthur M. Master, M.D., New York

10:05 A.M. Sodium Tolerance of Digitalized Cardiacs as a Method of Evaluating Diuretics and as a Guide to Diuretic Therapy
Solomon Fisch, M.D., Ph.D., Robert J. Sperber, M.D. (by invitation), Bennett P. Lustgarten, M.D. (by invitation), Ambrose P. Boyle, M.D. (by invitation), and Arthur C. DeGraff, M.D., New York

10:20 A.M. Clinical Experience With Quinethazone, an Orally Effective Diuretic Agent
Robert H. Seller, M.D. (by invitation), Morton Fuchs, M.D., and John H. Moyer, M.D., Philadelphia

10:35 A.M. Intermission

Vice-President Joseph C. Edwards, M.D., Presiding

10:45 A.M. The Paradoxical Effect of Chlorothiazide and/or Spironolactone in Patients
With Diabetes Insipidus
Herbert S. Kupperman, M.D., Ph.D., and Jeanne A. Epstein, M.D. (by invitation), New York

11:00 A.M. Study of p-Bromphenylindandione, a New Anticoagulant
*Mark M. Singer, M.D. (by invitation), Solomon Fisch, M.D., Ph.D., and Arthur
C. DeGraff, M.D., New York*

11:15 A.M. Anticoagulant Prophylaxis Against Pulmonary Embolism: A Cause of Recurrent Left Ventricular Failure
Benjamin Manchester, M.D. (by invitation), Washington, D. C.

11:30 A.M. Evaluation of the Antiarrhythmic Properties of Antazoline
*S. R. Kline, M.D. (by invitation), L. S. Dreifus, M.D. (by invitation), A. N.
Brest, M.D. (by invitation), and W. Likoff, M.D. (by invitation), Philadelphia*

11:45 A.M. Hypocholesteremic Agents: A Comparative Study
*Philip Lisan, M.D. (by invitation), Wilbur Oaks, M.D. (by invitation), Sheldon
Bender, M.D. (by invitation), and John H. Moyer, M.D., Philadelphia*

President-Elect George C. Griffith, M.D., Presiding

2:00 P.M. The Seventh Oscar B. Hunter Memorial Award in Therapeutics
Hattie E. Alexander, M.D., New York

2:45 P.M. Complications of Total Cardiopulmonary Bypass in Open Heart Surgery
*Gerald H. Pratt, M.D., William Wolff, M.D. (by invitation), John Comer, M.D.
(by invitation), David Barr, M.D. (by invitation), and Stanley Giannelli, M.D.
(by invitation), New York*

3:00 P.M. Intermission

Vice-President Adrian Lambert, M.D., Presiding

3:10 P.M. Five Years' Experience With Chemotherapy of Metastatic Choriocarcinoma
and Related Trophoblastic Disease in Women
Roy Hertz, M.D. (by invitation), Bethesda

3:30 P.M. Cooperative Studies in Cancer Chemotherapy
Julius Wolf, M.D., New York

3:50 P.M. Precision Radiation Surgery as Applied to Bladder Carcinoma
Thomas H. Johnson, M.D., New York

4:10 P.M. Present Status of Perfusion in Cancer Chemotherapy
Adrian Lambert, M.D., New York

Saturday, June 24

Chairman of the Council William B. Rawls, M.D., Presiding

9:00 A.M. Therapy of Periodic Disease
Hobart A. Reimann, M.D. (by invitation), Philadelphia

9:15 A.M. The Action of Oxyphencyclamine Hydroxyzine on Gastric Acid Secretion With Reference to the Use of Intragastric Electrodes
John M. Gambescia, M.D. (by invitation), Philadelphia

9:30 A.M. Management of Fatigue: A Physiological Approach
Daniel L. Shaw, Jr., M.D. (by invitation), Murphy A. Chesney, M.D. (by invitation), I. Frank Tullis, M.D. (by invitation), and H. P. K. Agersborg, Jr., Ph.D. (by invitation), Philadelphia

9:45 A.M. Preliminary Observations on the Effect of Serotonin Inhibitors in Clinical Medicine
Arthur L. Scherbel, M.D., Cleveland

10:00 A.M. Clinical Pharmacology of Guanethidine
Albert N. Brest, M.D. (by invitation), Paul Novack, M.D. (by invitation), and John H. Moyer, M.D., Philadelphia

10:15 A.M. Evaluation of a New Treatment in Patients With Schizophrenia and Involutional Depressions
M. J. Miller, M.D. (by invitation), J. Dennehy, M.D. (by invitation), R. Shettel, M.D. (by invitation), D. M. Hand, M.D. (by invitation), and H. T. Fiedler, M.D. (by invitation), Philadelphia

10:30 A.M. Intermission

Vice-President Robert W. Oblath, M.D., Presiding

10:40 A.M. Asian Influenza: A Localized Epidemic in a Large Closed Population
William P. Boger, M.D., and Jack W. Frankel, Ph.D. (by invitation), Norristown

11:05 A.M. Quantitation of the Fibrinolytic System in Man
Paul W. Boyles, M.D. (by invitation), William H. Meyer, M.D. (by invitation), Jack Graff (by invitation), and Paul U. Gerber, Jr., M.D. (by invitation), Miami

11:30 A.M. The Tenth Annual Lewis Harvie Taylor Lecture: The Newer Penicillins
Harry F. Dowling, M.D. (by invitation), Chicago

R. Emmet Kelly, M.D., Presiding

2:00 P.M. Hypercalcemia: A Clinical Spectrum
Noble David, M.D. (by invitation), John V. Verner, Jr., M.D., and F. L. Engel, M.D. (by invitation), Durham

2:15 P.M. Effect of Nalorphine on Pupil Diameter
Henry W. Elliott, M.D., and E. Leon Way, M.D. (by invitation), San Francisco

2:30 P.M. The Use of Hydroxychloroquine in the Treatment of Rheumatoid Arthritis
Charles W. Denko, M.D., Ph.D. (by invitation), Columbus

2:45 P.M. The Effect of Varying Doses of the Steroids Upon the Lee-White and the Heparin Retarded Clotting Time
William B. Rawls, M.D., New York

3:00 P.M. The Future of the American Therapeutic Society
President-Elect George C. Griffith, M.D.

Panel Discussion. "The Population Explosion: What Can the Physician Do About It?"
Moderator: Howard C. Taylor, Jr., M.D. (by invitation), New York

3:10 P.M.   Population Density and Medicosocial Problems as Indications for Conception Control
Alan F. Guttmacher, M.D. (by invitation), New York

3:25 P.M.   Factors Involved in Patient Acceptance of Methods Used in Population Control
Mary Steichen Calderone, M.D. (by invitation), New York

3:40 P.M.   The Use of Steroid Hormone Therapy for Oral Contraception
Edward T. Tyler, M.D., Los Angeles

4:05 P.M.   Panel Discussion
Moderator: Dr. Taylor; Drs. Guttmacher, Calderone, and Tyler

4:45 P.M.   Business Meeting

The anticoagulants

Although the anticoagulants have been in clinical use for only two decades, they are now established as unique preventive agents. These drugs do not lyse clots but prevent the formation of clots.[3] When given in effective doses they may prevent thromboembolic phenomena and subsequent death. Since they have few other pharmacologic effects, they are examples of highly selective drugs.

The coumarins

Because the coumarins are effective orally and are relatively inexpensive, they have proved practical for long-term use. The synthetic compounds now available had their origin in veterinary medicine.

In the 1920s a new malady of cattle involving fatal bleeding appeared.[7] The disorder was traced to stacks of decomposing sweet clover hay. Roderick in 1931 emphasized that the decreased coaguability of the blood was due to a deficit of prothrombin (factor II). More recently, the coumarin derivatives have been shown to have more than a hypoprothrombinemic effect.[1] They also depress factors VII, IX, and X.

Link and his co-workers isolated the substance from the hay and identified it chemically as bishydroxycoumarin (Fig. 1).

Bishydroxycoumarin (Dicumarol) was released for clinical use in 1940. Since then, a variety of related agents have been proved effective (Table I). They all act in essentially the same way to prevent manufacture of coagulation proteins. Vitamin K_1 (Mephyton) counteracts those effects.

An informative article comparing coumarin derivatives points out that there is no striking difference in onset of effect or smoothness of control.[12] The agent of choice is the established drug with which the doctor is most familiar.

The coumarins are usually given orally. Warfarin, however, can be given parenterally. The drugs are absorbed from the gastrointestinal tract, but absorption is slow, limited, and erratic and varies with the patient and the dose administered. The duration of action and patient sensitivity vary greatly from patient to patient, and cumulative action may be a complication. Furthermore, the mild hypoprothrombinemic effects of aspirin may complicate the expected response as may other agents such as bowel-sterilizing antibiotics.

With most of these agents in common doses now used, maximal effects are seen usually only after 24 to 48 hours, although the drug can be demonstrated to be present prior to the maximal effect on the blood-clotting scheme. There is no difference in the speed of action after intravenous or oral dose. The size of the dose determines the maximal decrease in the clotting proteins and the duration of effect. The aim of therapy is to decrease the prothrombin activity as determined by the one stage Quick test to between 15 and 25 per cent of normal.

The fate of these compounds is relatively unknown. They are almost completely metabolized by a slow degradation process. With clinically effective doses of bishydroxycoumarin and phenprocoumon, the prothrombin activity usually does not return to normal for 1 to 2 weeks after stopping the drug.

The coumarins cause bleeding in patients who have some predisposing cause such as a duodenal ulcer. If the Quick prothrombin test result drops below 10 per cent of normal, clotting time is altered and patients are very likely to bleed from minor injuries. The coumarins cause no liver damage. In the doses used in

man, no toxic effects are seen such as damage to small blood vessels. Large doses of these agents in animals can be demonstrated to have this effect. Occasionally, mild gastrointestinal symptoms such as nausea, vomiting, and diarrhea have been reported. Skin rashes and agranulocytosis and hepatitis have occurred in patients taking phenindione.[6] Side effects are unusual, and in general there are minimal idiosyncratic reactions from these drugs.

Heparin

Heparin was discovered in 1916 by J. McLean, a second year medical student working at Johns Hopkins. Howell and Holt described the characteristics of this anticoagulant and established its identity as a polysaccharide. Because of its high concentration in the liver, it was named "heparin." Important contributions in improved methods of extractions were made by Charles and Scott. The drug is still prepared commercially by extracting it from animal tissues such as beef lung. Jorpes and his collaborators made rapid advances in elucidating the chemistry of the compound, but as yet the chemical constitution of heparin has not been definitely established (Fig. 2). It is known to be a naturally occurring mucoitin-polysulfuric acid. It consists of equal parts of hexuronic acid and acetylated glucosamine.

The degree of polymerization in heparin is unknown, but the molecular weight has been estimated to be approximately 20,000. Heparin is a strong acid—the strongest organic acid found in the body.

A principal characteristic of the molecule is its strong electronegative charge, which is most likely the basis for its anticoagulant properties. The charge affects the clotting proteins by preventing their interaction, which is coagulation.

Howell noted the low toxicity of heparin and early suggested that it might be useful in the treatment of disorders of coagulation. However, it was not until 1937 that it was first employed clinically by Murray of Toronto and Crafoord in Sweden.

The drug has in vitro as well as in vivo effect on coagulation. It must be given parenterally, and when given intravenously some action is seen immediately, its

Table I

Drug	Proprietary name	Peak effect	Recovery	Initial dose	Maintained dose	Route
Aceno-coumarin	Sintrom	24-48 hr.	48 hr.	16-24 mg.	2-10 mg.	Oral
Anisindione	Miradon	48-72 hr.	24-72 hr.	300 mg.	75-100 mg.	Oral
Bishydroxy-coumarin	Dicumarol	24-72 hr.	7-9 days	200-300 mg.	25-100 mg.	Oral
Ethyl biscou-macetate	Tromexan	18-24 hr.	48 hr.	1.5-1.8 Gm.	0.6-0.9 Gm.	Oral
Phenindione	Danilone, Hedulin, Indon	18-24 hr.	24-48 hr.	200-300 mg.	50-100 mg.	Oral
Phenpro-coumon	Liquamar	36-48 hr.	7-14 days	21-30 mg.	0.75-6 mg.	Oral
Warfarin sodium	Coumadin sodium, Prothrom-adin	12-18 hr.	5-7 days	10-50 mg.	2.5-10 mg.	Oral, intra-muscular, intra-venous
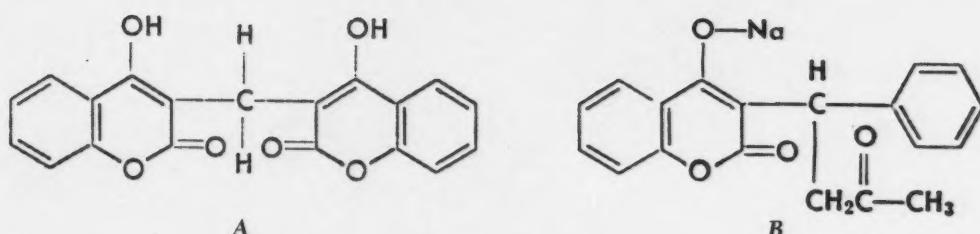
Fig. 1. Structural relationship of warfarin sodium to bishydroxycoumarin. *A*, Bishydroxycoumarin (3,3'-methylenebis[4-hydroxycoumarin]). *B*, Warfarin sodium (3-[α-acetonylbenzyl]-4-hydroxycoumarin).
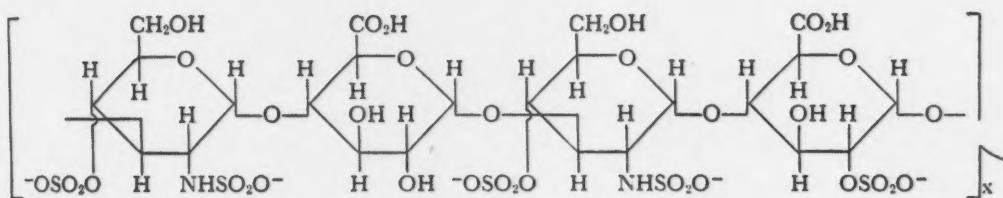
Fig. 2. Tetrasaccharide unit of heparin.

peak effect occurring in 10 minutes. It is thought that this time is required for full combination with the clotting proteins. Heparin effect is measured by some therapists by its action on the clotting time. Their aim is to increase a standard clotting time two to three times the normal value at 2 hours after intramuscular administration and to be sure the clotting time is at least one times normal immediately before the next drug administration. Other therapists give fixed amounts and do not gauge the doses by clotting times.

The metabolism of heparin is not fully understood. About 25 per cent of a given dose appears in the urine in active form. Another portion is depolymerized to an inactive form which is excreted in the urine. The existence in the liver of a heparin-inactivating enzyme has been established. Even after large doses given intravenously, the effect is gone within a few hours. Agents such as protamine sulfate in milligram for milligram dose ratio quickly counteract heparin effect.

The most important adverse effect from heparin is hemorrhage. It is usually not observed in the patient who does not have some predisposing cause. While anaphylactic reactions to heparin have been reported, such major sensitivity is rare. Rash and fever are seen at times. If heparin is given subcutaneously repeatedly, as many as 50 per cent of the patients may develop a local reaction of the wheal and flare type. Reactions to a certain brand of heparin may be avoided by selecting a different brand. Another complication of heparin therapy is alopecia. This is uncommon and usually disappears within approximately 6 months after cessation of therapy. Heparin substitutes have caused alopecia in high percentages of the cases tried. Because of toxicity, no heparin substitute has as yet gained clinical acceptance.

Therapeutic uses

Unfortunately, enthusiasts have given the impression that these agents are panaceas for thromboembolic disease. Actually, few examples of striking effectiveness of the anticoagulants have been proved. On the other hand, it is well known that severe bleeding and even death may occur even with reasonably

careful use of these agents. No completely satisfactory method of safely regulating coumarin dosage is available generally. A new test called the "thrombotest"[10] holds promise of better control.

Many types of clinical conditions have been thought to be indications for anticoagulant therapy. Discussions of use in rheumatic heart disease, thrombophlebitis, etc., are considered in Newcomb's article on anticoagulants.[9]

Because it is believed that the therapeutic usefulness of these agents (the risks balanced against the benefits) can be carefully evaluated exclusively through a controlled trial, only such information on therapeutic usefulness will be stated here. Striking reduction of pulmonary embolism with coumarin agents has been proved in patients who are recovering from femur fractures for which they have been immobilized.[13] Similarly, marked reductions in the number of thromboembolic phenomena have been shown in patients with congestive heart failure.[4] Patients with angina pectoris without myocardial infarction or congestive heart failure have had an important reduction in mortality on phenindione when the prothrombin-proconvertin test was kept at 20 per cent of normal.[2] Less striking but clear improvement has been shown in the infarct recurrence rate and mortality after a myocardial infarction in men under 55.[11] Critical trials with anticoagulants in patients with cerebral thrombosis did not seem to show practical benefit in these cases.[5,8] The other claims for usefulness have not been supported yet by satisfactory evidence. Since these agents are potentially harmful a critical look is proper before therapy is undertaken!

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articles to appear in early issues

Diseases of medical progress

Robert Harlan Moser, Major, MC, USA, Honolulu, Hawaii

Clinical and experimental observations with methiodal, an absorbable myelographic contrast agent

J. Paul Harvey, Jr., M.D., Robert F. Freiberger, M.D., and Gerhard Werner, M.D., New York, N. Y.

Anesthetic time/dose curves. III. The interaction of thiopental and oxymorphone during surgical anesthesia

M. Keeri-Szanto, M.D., M. Knaff, M.D., and Y. Rondeau, M.D., Montreal, Que.

The effect of methionine sulfoximine in man

Irwin H. Krakoff, M.D., New York, N. Y.

An assessment of the responses to drugs acting on the central nervous system

Cedric W. M. Wilson, M.D., Ph.D., and Pamela M. Huby, M.A., Liverpool, England

Clinical trials based on patients' preferences

J. D. Acland, B.M., Sheffield, England

Effects of vanillic acid diethylamide in man

Murray J. Miller, M.D., B. Marvin Hand, M.D., and J. Antrim Crellin, M.D., Philadelphia, Pa.

Symposium on the experimental pharmacology and clinical use of antimetabolites. Part IX. The pyrimidine and purine antagonists

Joseph H. Burchenal, M.D., and Rose Ruth Ellison, M.D., New York, N. Y.